Determination of polar pesticide metabolites in aqueous matrices by LC-MS and investigation of occurring matrix effects

Dissertation

zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften

– Dr. rer. nat. –

vorgelegt von

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geboren in Bielitz (Polen)

Fakultät für Chemie

der

Universität Duisburg-Essen

2013

Die vorliegende Arbeit wurde im Zeitraum von Juni 2008 bis April 2013 im Arbeitskreis von Prof. Dr. Torsten C. Schmidt am IWW Rheinisch-Westfälischen Institut für Wasser, einem An-Institut der Universität Duisburg-Essen, durchgeführt.

Tag der Disputation: 26.06.2013

Gutachter: Prof. Dr. Torsten C. Schmidt

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Vorsitzender: Prof. Dr. Mathias Ulbricht

Acknowledgements

I would like to thank the following persons for without whose help and expertise this thesis would not have been possible.

My greatest thanks are devoted to Prof. Dr. Torsten C. Schmidt, Dr. Friedrich Werres and Dr. Peter Balsaa for the allocation of the interesting scientific topic as well as for their continuous helpful advice during this thesis.

My special thanks go to all my nice colleagues at the IWW Water Centre and Department of Instrumental Analytical Chemistry at university Duisburg-Essen for suggestions, support and inspiring discussions.

Finally, I wish to thank my parents and Jinny, for their patience, understanding and support during the study.

Abstract

The application of numerous pesticides in agriculture and municipal areas several times a year, leads to the infiltration of pesticides into the topsoil via leaching and drainage processes. Furthermore, partial chemical or biological degradation lead to the formation of pesticide metabolites. The formed metabolites are typically more mobile and persistent than the precursors and contribute to a contamination of ground water resources. During conventional water treatment, those polar metabolites often cannot be effectively removed. Additional risks may occur when these substances are chemically modified by oxidative processes like ozonation or disinfection in drinking water treatment, forming products of toxicological concern. Monitoring and maintaining water quality during drinking water treatment requires reliable and sensitive analytical methods in order to respond directly, if any significant changes in water quality occur. At present, the analysis of polar pesticide metabolites is usually carried out by liquid chromatography tandem mass spectrometry (LC-MS/MS). The determination of trace amounts of organic constituents in water often requires large volume direct injection (LVDI). The application of a large amount of sample matrix leads to a significant influence on the electrospray ionisation (ESI) process which in turn affects correct quantification. Major objectives of this study were therefore the development and optimisation of LC-MS/MS methods for quantification of pesticide metabolites in trace level and examination of matrix influences on ESI. In this context, the chromatography was optimised for the requirements for LVDI and separation of polar pesticide metabolites from sample matrix. To reduce or compensate matrix effects, various strategies have been developed, optimised and examined for their suitability in the specific water matrix. Subsequently, with the developed analytical methods the occurrence of pesticide metabolites in surface water, ground water and drinking water has been investigated in the area around the Rhine and Ruhr (North Rhine-Westphalia).

For N,N-dimethylsulfamide (DMS) – a metabolite of the fungicide tolylfluanid – it is already known that during the drinking water treatment with ozone the carcinogenic N-nitrosodimethylamine (NDMA) is formed. Furthermore, it is known that the disinfection with hypochlorite degrades DMS completely, however it was unknown which chlorination transformation products are formed. Therefore, a further objective of this work was to detect and characterise potential chlorination transformation products, which were formed during the chlorination with hypochlorite.

Kurzfassung

Die langjährige Anwendung von Pflanzenschutzmittelwirkstoffen (PSM-Wirkstoffen) in der Landwirtschaft und auf kommunalen Flächen führt durch Versickerung oder Abschwemmung der PSM-Wirkstoffe zu einer Beeinträchtigung der Grundwasserressourcen. Dabei werden nicht nur die PSM-Wirkstoffe selbst, sondern oft ihre biologischen oder chemischen Metaboliten im Grundwasserleiter nachgewiesen, da sie zumeist eine erhöhte Wasserlöslichkeit und Persistenz aufweisen. Hinzu kommt, dass die PSM-Metaboliten aufgrund ihres polaren Charakters während der Wasseraufbereitung nicht immer effektiv entfernt werden und bei Einsatz von oxidativen Trinkwasseraufbereitungsprozessen sogar toxikologisch bedenkliche Stoffe bilden können. Daher werden zur Überwachung der Wässer im Vorfeld der Trinkwassergewinnung schnelle, sichere und empfindliche Analysenmethoden benötigt, um unverzüglich auf Veränderungen der Wasserqualität reagieren zu können. Die Analytik von polaren PSM-Metaboliten erfolgt inzwischen meist mit der Flüssigchromatografie-Tandemmassenspektrometrie (LC-MS/MS). Um den Anforderungen der Bestimmung im Spurenbereich gerecht zu werden, erfolgt häufig die Direktinjektion großer Probenvolumen (LVDI). Hierbei gelangen nicht nur die Zielsubstanzen in das Massenspektrometer, sondern häufig auch störende, in der Wassermatrix enthaltene Komponenten, die mitunter zu einer deutlichen Beeinflussung der Ionisierung und dadurch Beeinträchtigung der präzisen Quantifizierung führen. Neben der Entwicklung und Optimierung von LC-MS/MS Methoden für die Quantifizierung der PSM-Metaboliten im Spurenbereich bestand daher ein wichtiges Ziel dieser Arbeit darin, die Auswirkung des Matrixeinflusses zu untersuchen. In diesem Zusammenhang wurde insbesondere die Chromatografie optimiert, um die Anforderungen an die LVDI und Trennung der polaren Metaboliten von der matrix zu erfüllen. Um den Matrixeinfluss zu reduzieren bzw. zu kompensieren, wurden verschiedene Strategien entwickelt, optimiert und auf ihre Eignung in der spezifischen Wassermatrix untersucht. Abschließend wurden die entwickelten analytischen Verfahren zum Monitoring von Oberflächen-, Grund- und Trinkwässern eingesetzt und der Eintrag von PSM-Metaboliten im Gebiet um Rhein und Ruhr untersucht.

Ein weiteres Ziel der Arbeit war die Detektion und Charakterisierung von Nebenprodukten des Fungizid-Metaboliten N,N-Dimethylsulfamid (DMS), die bei der Chlorierung von DMS-haltigen Wässern entstehen können. Aus der Literatur war hierzu bisher nur bekannt, dass beim Einsatz von Ozon im Rahmen der Trinkwasseraufbereitung in DMS-haltigen Rohwässern das kanzerogene N-Nitrosodimethylamin gebildet, während bei der Chlorung des Trinkwassers mittels Hypochlorid das DMS vollständig abgebaut wird.

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List of Abbreviations and Symbols

µg	micro gram(s)
µm	micro meter(s)
3D	three dimensions
a	year(s)
amu	atomic mass unit
AP	atmospheric pressure
BVL	Federal Office of Consumer Protection as well as Food Safety
CID	collision-induced dissociation
CODA	component detection algorithm
D_6DMS	Isotopically labeled DMS
Da	Dalton
DIN	German Institute for Standardisation
DMA	dimethylamine
DMC-Cl	chlorinated DMS
DMH	dimethylhydrazin
DMS	N,N-dimethylsulfamide
DOC	dissolved organic carbon
DPC	desphenyl-chloridazon
EC	European Council
EC_{50}	mean effective concentration
EIC	extracted ion chromatograms
EQS	environment quality standards
ESA	sulfonic acid
ESI	electrospray ionisation
eV	electron volt
h	hour(s)
HPLC	high performance liquid chromatography
HR	high-resolution
HRIV	health related indication values
IS	isotopically labeled internal standard
k	retention factor
K_{ow}	octanol-water partition-coefficient

L	litre
LC	liquid chromatography
LC_{50}	mean lethal concentration
LD_{50}	mean lethal dose
LLE	liquid-liquid-extraction
LOD	limit of detection
Log D	distribution-coefficient
Log P	partition-coefficient
LOQ	limit of quantification
LVDI	large volume direct injection
M	Molar mass
m/z	mass to charge ratio
MDPC	methyl-desphenyl-chloridazon
mg	milligram(s)
min	minute(s)
mm	millimetre(s)
mmol	millimol(s)
MS	mass spectrometry
ms	millisecond(s)
MS/MS	tandem mass spectrometry
n	number
NDMA	N-nitrosodimethylamine
OA	oxal acid
PA	proton affinity
pH	potentia hydrogenii
pKa	acid dissociation constant
PPP	plant protection products
Q	quadrupole
QqQ	triple quadrupole mass spectrometer
Q-TOF	quadrupole time-of-flight
R	coefficient of correlation
%RSD	relative standard deviation
s	second(s)
S/N	signal-to-noise

SAM	standard addition method
SDMH	symmetrical-dimethylhydrazin
SIR	selected ion monitoring
SPR	solid-phase-extraction
SRM	selected reaction monitoring
STAD	standard addition procedure
TIC	total ion chromatograms
TRIS	tris(hydroxymethyl)aminomethane
UBA	Federal Environmental Agency
UDMH	unsymmetrical-dimethylhydrazin
UPLC	ultra performance liquid chromatography
V	volt
v/v	volume to volume
w/w	mass to mass

Chapter 1. General introduction

1.1 Pesticide metabolites

Currently, about 250 pesticide active ingredients (in the following called pesticides) in more than 900 different commercial plant protection products (PPP) are approved in Germany (status 2010) [1]. The spectrum of pesticides comprises mainly herbicides (50%) followed by fungicides (23%), insecticides (13%), and growth regulators (8%) [2]. The annual sales volume of PPP has increased since 1994 until in recent years a constant annual sales volume of about 35,000 tons of PPP has been reached in Germany [2]. In the European Union alone, more than 200,000 tons of pesticides are used annually [3]. Moreover, the spectrum of pesticides has changed in the last 50 years to more polar and more degradable substances [4]. PPP are primarily utilised in agriculture (80%); other fields of application are the treatments of railway tracks, municipal areas, and private use (20% in total). The application is carried out several times per year and over large areas. Especially when applied incorrectly, pesticides may enter surface water and ground water [5]. The most important ways for pesticides to enter the surface water is via run-off, and the ground water via drainage by seepage water [5].

The evaluation of the contamination situation of ground water and surface waters by pesticides in Germany is difficult because no nationwide systematic monitoring program exists [6]. However, the following statements represent an approximate overview. In a nationwide interview of drinking water suppliers, 38% positive detection of pesticides in raw water (ground water, well water and bank filtrate) in the period 2000 – 2006 was specified. 50% of the pesticides detected were ones that are no longer allowed, 43% were approved and 7% were pesticide metabolites [7]. The Working Group on water issues (LAWA) 2011 report, which summarised data from the Federal Environmental Agency (UBA), shows that the number of positive detections (concentrations higher than 0.1 µg/L) of all measurement points (13,000) in the period 1990 - 2008 significantly decreased from 8.6% to 4.6% [8]. These data show in general, the pollution of different water bodies is caused by pesticides but

not its metabolites. Only 5% of the pesticides reach the target organism or are deposited on the soil, while a not negligible part of the pesticides are metabolised by biological or chemical processes [9]. The metabolites are usually more polar and persistent than the original substance, with the result that cannot be effectively removed during water treatment processes, e.g. bank filtration or activated carbon adsorption. Additionally, if oxidative water treatment steps are used, substances of toxicological concern may be formed. This is true for the case of N,N-dimethylsulfamide (DMS), a polar metabolite of the fungicide tolylfluanid that was approved in 1974. DMS has been detected for the first time in significant concentrations in ground water in different regions of Germany at the end of 2006 [10]. The use of certain operational settings of an ozonation process during the water treatment can lead to the conversion of already small amounts of DMS to the carcinogenic N-nitrosodimethylamine (NDMA) [10]. As a consequence of these results, the outdoor application of tolylfluanid was banned in early 2007 [10]. At the same time high concentrations of desphenyl-chloridazon, known from lysimeter tests – which are carried out in the context of pesticide authorisation procedure and the results in general are not accessible to the public – up to 10 µg/L were found in many ground water samples. Also, another hitherto unknown degradation product, methyl-desphenyl-chloridazon (MDPC), was detected and characterised in water samples from Baden-Württemberg [11]. Both are metabolites of the herbicide chloridazon, which has been used since 1964 for sugar beet, beetroot and onion cultivation [12]. These results show the limited knowledge about pesticide metabolites in groundwater, since unexpected metabolites have recently been found for certain parent pesticides that were commercialised over the last 30 to 40 years. Within the framework of approval processes for pesticide active ingredients from lysimeter tests, numerous pesticide metabolites are known. However, up to now there was not much information publicly available about studies involving lysimeter tests. Because of positive findings of more hitherto unknown metabolites and pressure of the public, the Federal Office of Consumer Protection as well as Food Safety (BVL) and the Federal Environment Agency (UBA) published data in 2008 about so-called non-relevant metabolites – that were detected in high concentrations of 1 - 10 µg/L – during lysimeter tests (Table 1-1) [1, 13]. Metabolites are called non-relevant when they are free from original toxicities of the pesticide and show no genotoxic properties that are

considered unacceptable [14]. Previous sampling campaigns of individual pesticide metabolites show the frequent occurrence of positive detections in ground water and drinking water in entire Germany [13, 15, 16]. Currently, neither regionally nor nationally a systematic monitoring of all previously published pesticide metabolites exists for detecting the concentrations in different water compartments. In addition, no knowledge about the behaviour of the pesticide metabolites during drinking water treatment exists, especially when oxidative treatment processes are used. Consequently, it is important to monitor these substances in the aquatic environment with reliable and sensitive analytical methods to enable an immediate response if changes occur in water quality. Additionally, the behaviour of the metabolites in oxidative drinking water treatment should be investigated.

Table 1-1: Overview of the investigated pesticide metabolites in this work, their log P values (simulated [17]), health related indication values (HRIV) and maximum concentrations in lysimeter tests [1, 18-20].

Nr.	Metabolite	Molar mass (g/mol)	Application rate during lysimeter tests (kg/h)	Maximum concentrations in lysimeter water (µg/L)	Partition coefficient (log P)	Health orientation value (µg/L)
1	N,N-dimethylsulfamide	124.2	--	--	-1.54	1
2	desphenyl-chloridazon	145.5	2.50	40.6	-0.78	3
3	methyl-desphenyl-chloridazon	159.6	2.50	2.1	-0.55	3
4	2,6-dichlorbenzamid	190.0	--	94.0	2.03	3
5	flufenacet-OA	225.2	--	--	1.95	--
6	flufenacet-M2	275.3	0.48	1.6	1.19	1
7	dimethenamid-P-M23	271.3	1.39	1.0	2.34	1
8	dimethenamid-P-M27	343.4	1.39	4.0	1.58	1
9	dimetachlor-CGA369873	265.3	1.50	2.3	1.44	1
10	dimetachlor-CGA354742	323.3	1.50	35.1	1.25	3
11	dimetachlor-CGA50266	251.3	1.50	36.2	2.00	3
12	chlorthalonil-M5	268.5	2.50	1.4	2.29	3
13	chlorthalonil-M12	329.6	0.99	10.3	-0.68	3
14	metazachlor-BH479-4	273.3	1.00	21.4	2.05	1
15	metazachlor-BH479-8	323.4	0.96	17.0	0.35	3
16	metazachlor-BH479-9	349.4	0.96	1.3	1.01	3
17	metazachlor-BH479-11	305.4	0.96	2.5	1.19	3
18	metazachlor-BH479-12	303.3	0.96	3.6	1.09	3
19	S-metolachlor-CGA351916	279.4	1.25	16.3	2.88	3
20	S-metolachlor-CGA-380168	351.4	1.25	28.0	2.11	3
21	S-metolachlor-CGA368208	279.3	1.25	7.8	1.88	1
22	S-metolachlor-CGA357704	279.3	1.25	5.1	--	1
23	S-metolachlor-NOA413173	373.3	--	3.0	--	1
24	metalaxyl-M-CGA62826	265.3	0.16	6.9	1.97	1
25	metalaxyl-M-CGA108906	295.3	0.16	1.9	1.12	1
26	quinmerac-BH518-2	251.6	0.24	2.4	1.51	1
27	tritosulfuron-BH635-4	353.3	0.05	1.0	0.25	1
28	dimoxystrobin-505-M08	356.4	0.25	2.4	0.70	3
29	dimoxystrobin-505-M09	356.4	0.25	2.0	0.70	3

1.2 Instrumentation

The application of liquid chromatography coupled with electrospray ionisation tandem mass spectrometry LC-ESI-MS/MS has increased steadily since the 1990s in the environmental field and is at present the method of choice predominantly for the determination of polar compounds such as pesticides in different water bodies [21-23]. To arrive at sufficiently low limits of detection that are needed for environmental quality standards given by the European Council Directive for drinking water (98/83/EC), the compounds are often enriched prior to analysis [24, 25]. However, standard sample preparation methods such as solid-phase extraction or liquid-liquid extraction are time consuming and not effective due to the high polarity of many compounds with octanol/water partition-coefficient (K_{ow} or log P) < 3, consequently leading to low recoveries. Therefore, large-volume direct-injection (LVDI) LC-MS is frequently used to fulfil the requirements for a quantification of such substances on the trace level [11, 23]. With the use of LVDI, not only the target analytes enter directly into the mass spectrometer, but also many environmental water constituents such as inorganic salts or humic substances (summarised under the term sample matrix). Especially if these co-elute with the target analytes, they affect the ionisation efficiency (ion suppression/enhancement) during the measurement, the so-called matrix effect [26-31]. This matrix effect occurs very often during quantitative analysis using LC-ESI-MS/MS and affects its accuracy, precision and limit of detection [32]. It is therefore important to examine the influence of matrix effects when using LC-ESI-MS/MS for quantification in complex samples or in different environmental matrices. In the following, the different methods and techniques are described in detail separately for chromatography, mass spectrometry, and matrix effects.

1.2.1 Chromatography

High performance liquid chromatography (HPLC) has been used successfully for quantification of polar substances over the past 30-plus years in laboratories worldwide [33]. The most common separation phase, which is reversed-phase, is not optimal for polar substances but has become well-established in HPLC because of the simple handling involved. In recent years, significant technological advances

have been made for reversed-phases with regard to particle chemistry and decreased particle size as well as for HPLC equipment. This has led to an enormous increase in the selectivity (wider choice of stationary phases) and especially in the sensitivity and separation speed [33, 34]. The ultra performance liquid chromatography (UPLC) equipment uses columns with smaller inner diameter (≤ 2.1 mm) and particle size (< 2 µm) that leads to an increased resolution, sensitivity and fast separation. By using these columns (2.1 x 50 mm x 1.7 µm) in comparison with conventional columns (4.6 x 50 mm x 5 µm), at the same chromatographic conditions the sensitivity is increased by a factor of 3 to 5 and the run time is decreased by a factor of 5 at the same selectivity [35, 36]. A further important development is the introduction of ethylene-bridge-hybrid technology that leads to an increase of mechanical stability at higher pressure, extended pH range, and hydrolytic stability [33]. Additionally, embedded polar groups in the reversed-phase increase the selectivity and separation efficiency for polar substances [37]. The UPLC system for using sub micro particles must be especially designed to withstand higher system pressures, because the system involves a high pressure fluidic binary pump, which works at pressures up to 1000 bar. Additionally, the dead volume on such systems was decreased (to approximately 50 µL) to allow a fast gradient separation. The use of reversed-phase and columns with small inner diameter requires optimisation of chromatography for the application of a LVDI of polar substances, because even a small volume leads to overloading the column and peak tailing. For the optimisation of chromatography, new stationary phases and the UPLC have further advantages. For example, the hydrolytic stability of the new column materials allows a nearly 100% aqueous initial gradient in combination with a fast increase of the organic modifier content in the mobile phase, and polar embedded groups lead to the focusing of polar substances on the head of the column (on-column focusing) [38, 39]. This technique leads to sharper (narrower) and higher peaks by using LVDI for polar compounds. In summary, these new approaches in method development for polar pesticides in the environmental area were needed for the quantification at trace level down to low ng/L range and were applied in this work.

1.2.2 Mass spectrometry

The LC coupled with atmospheric pressure ionisation mass spectrometry (MS or tandem MS (MS/MS)) is a powerful tool for the rapid detection, structure elucidation, and quantification of polar substances in aqueous solutions [40-44]. Commonly used tandem mass spectrometers are triple quadrupole (QqQ) or quadrupole time-of-flight (Q-TOF) for quantification or structure elucidation, respectively. The triple quadrupole is a mass spectrometer composed of three quadrupoles (Q), commonly called MS^2, MS/MS or QqQ. It contains an ion source, Q1 and Q3 as mass analysers and Q2 as collision chamber. The Q1 mass analyser filters ions to select a specific mass/charge ratio (m/z); these ions are fragmented by inert gas molecules (collision-induced dissociation (CID) typically by argon or nitrogen) within Q2, and their fragment ions are scanned by Q3 before reaching the mass detector. This specific arrangement allows different specific measurement modes [45]. MS-scan and MS/MS-scan modes acquire full scan data for precursor or product ions. These modes are suitable for detection of compounds in a sample and structure elucidation by interpretation of fragmentation patterns. The non-selective nature of full-scan mass spectrometric data acquisition ensures that most ionisable substances are detected. However, the approach has the significant disadvantage of yielding a multitude of interfering data from the sample matrix. Therefore, subsequent data processing performed manually or computer assisted is needed to enable targeted detection of potential components from complex data. Other modes that can be employed, namely the selected-ion monitoring (SIR) and selected-reaction monitoring (SRM) modes are primarily used for quantification because of their sensitivity and selectivity [40, 46]. A Q-TOF mass spectrometer contains an ion source, a quadrupole MS followed by the TOF MS, which includes an ion pusher, reflectron, and detector [45]. The TOF MS allows very high mass resolution ($R = 2 \times 10^4$) readily resolving masses differing by 0.1 amu [45]. The exact masses together with the isotope pattern (specific natural isotopic distributions of the elements) are used for generating an empirical molecular formula, by using formula generator software that is helpful for the identification of unknown compounds [47]. However, MS data alone do not provide sufficient structural information necessary for a complete characterisation of compounds. Therefore, other analytical techniques, in silico tools [48] or mass databases [49], and finally the

confirmation using an authentic analytical standard enable unambiguous structural elucidation of substances [45].

The introduction of HPLC effluent directly into the MS - by transforming solution molecules from the liquid phase to the gas phase - is dependent on the source where the liquid is vaporised, and the molecules ionised prior to entering the MS. Electrospray ionisation (ESI) was used frequently, while atmospheric pressure chemical ionisation (APCI) or atmospheric pressure photoionisation (APPI) were used to a much lesser extent in the environmental area. Whereas the ESI method is mainly suitable for ionisation of polar substances, the APCI and APPI methods are used for substances of low polarity. APCI is ionisation in which the analytical sample is subjected to a corona discharge and APPI by using ultraviolet light [31, 50]. The ESI technique in positive and negative ionisation mode has been applied for acidic, basic and neutral compounds in the m/z range from 20 – 3000 Da (device-specific) [21, 40, 46]. During the ESI process, quasi-molecular ions $[M+H]^+$ in positive or $[M-H]^-$ in negative ionisation mode of the compounds are formed by strong charge in the mobile phase. The theory of the ESI process is presented in more detail in reviews [31, 51-54]. An important rule is that neutral compounds with a gas-phase proton affinity (PA) higher than that of ammonia (853.6 kJ/mol) can be ionised efficiently via a gas-phase proton transfer reaction in ESI, and components with lower PA (like many matrix constituents in environmental samples) may frequently suppress the ESI process [50]. Overall, the ESI process is complex and the efficiency of this process is dependent on several parameters like mobile phase flow rate, desolvation temperature, pH value of mobile phase, modifier, additives, and sample matrix (ions or non-volatile organic substances). The LC-ESI-MS/MS is a concentration dependent device, i.e. with a higher flow rate of mobile phase the detected compound concentration decreases. The desolvation temperature plays an important role, because of the evaporation process. It is generally assumed, that a higher temperature results in a higher intensity; however the optimal temperature is compound dependent. To improve the weak ionisation process, additives such as acid as proton donator for basic compounds in positive ESI mode, and bases as proton acceptors for acidic compounds in negative ESI mode are essential . Often, the best sensitivity in ESI is achieved when the analyte is ionised already in a liquid

phase by using acidic mobile phase for basic analytes, such as amines, (rule of thumb, pH two units below pKa of the analyte) and basic conditions for acidic analytes, such as carboxylic acids and phenols (pH two units above pKa of the analyte) [50]. However, the concentrations of these modifiers strongly influence the ionisation. This behaviour may differ in some cases, it is known that contrary to the standard expectation, basic analytes would be suppressed under acidic conditions and vice versa (this is referred as wrong-way-round ionisation [55]). Therefore, the investigation of two different pH values of the mobile phase is frequently helpful to obtain the best intensity. In this case it should be mentioned that a free selection of the mobile phase and additive composition is not possible since the optimal conditions for chromatography (buffer, pH value) frequently lead to suppression of ESI efficiency and vice versa. Therefore, often a compromise has to be made between ionisation and chromatographic separation efficiencies. This shows the complexity of the method development in LC-ESI-MS/MS. Due to the strong interplay of hereto described parameters, they should all be optimised during method development and were investigated in this work.

1.2.3 Compensation and reduction of matrix effects

The environmental samples (e.g., surface water) contain about 1 g/L salts and 3 mg/L dissolved organic constituents (humic and fulvic material). In contrast the target analytes are typically present within a concentration range of 0.0001 - 3 µg/L. By using the reversed-phase chromatography, especially the salts as environmental matrix components co-elute with the polar compounds [31]. The matrix components are in competition to the target analytes during the ESI process and thus lead to influences on the signal response (matrix effect) and thus accuracy of quantification [26-28]. To ensure an accurate quantification of the target analytes, different methods for reduction or compensation have been proposed in order to compensate the matrix effect and are routinely used. A very simple method is the dilution of the sample, however, the use of dilution is limited by the required limits of detection for the target compounds [56]. An alternative method is the use of matrix-matched samples for calibration, which have a similar composition regarding the matrix components but do not contain the target compounds [30, 57, 58]. Unfortunately, the composition of

natural water samples varies in a broad range, so that the application of matrix-matched samples for calibration is not useful. A widely used method is standard addition, which provides very accurate results [28, 31, 59]. In practice, however, this method needs both a time-consuming sample preparation and evaluation of the obtained results and is therefore not suitable to establish a fast as well as economic routine method. Using a deuterium or ^{13}C-labeled internal standard is a fast and precise method because the internal standard has nearly the same chemical properties as the target compound and is influenced by the matrix effects in a similar way [27, 31, 57, 59-62]. However, isotopically labeled internal standard (IS) are often expensive and not commercially available for new substances. The matrix effects can sometimes lead to great variations in the quantification of polar substances in environmental samples and were previously not systematically examined in the field of polar pesticide metabolites.

1.3 Scope of this study

Because of the occurrence of polar pesticide metabolites hitherto unknown to the public (UBA List) in aquifer and lack of knowledge about their behaviour during drinking water treatment, these substances should be monitored with appropriate analytical methods. The scope of this study was to develop sensitive and reliable analytical methods for the determination of pesticide metabolites (Table 1-1) in different water bodies. The developed methods should be economically applicable in a routine laboratory. For determination of polar pesticide metabolites on the trace level, the LVDI LC-ESI-MS/MS was applied. In detail, the reversed-phase chromatography was optimised for the use of LVDI and for separation of the polar metabolites from sample matrix. However, after the optimisation there was still a significant matrix effect. Therefore, the mass spectrometry was optimised by additives to enhance the sensitivity, and matrix effects were reduced or compensated by different methods. The developed analytical methods were validated and investigated with the specific sample matrix for their reliability. Subsequently, the analytical methods were used to investigate the occurrence of pesticide metabolites in different water bodies from the Rhine and Ruhr region of North Rhine-Westphalia (Germany).

Chapter 2 shows a determination method for the polar metabolite DMS in different water bodies. DMS was first detected in ground water in end 2006 and cannot be removed effectively by current water treatment processes such as bank filtration or activated carbon adsorption. Ozonisation converts small amounts of DMS into the highly potent carcinogenic N-nitrosodimethylamine (NDMA) with a health related indication values of 10 ng/L for drinking water. Therefore, the development of a sensitive and reliable method for the quantification of DMS was required. Due to the polarity of DMS, frequently used sample preparation methods such as solid-phase-extraction (SPE) and liquid-liquid-extraction (LLE) were not applicable. In the frame of this thesis, the approach of LVDI UPLC-ESI-MS/MS for the quantification of DMS was use. Matrix effects were compensated by using deuterated D_6DMS as IS. The difficult chromatographic separation and peak symmetry on reversed-phase of the polar DMS were investigated with different column phase types, dimensions and chromatographic parameters (temperature, gradient). Because of the influence of salts as a matrix constituent in the environmental samples on the ESI, a new approach was tested for determining matrix effects on the DMS signal. The matrix effects of the DMS signal were investigated with model samples that contain salt concentrations typically present in environmental samples. The reliability of this method was investigated in different real water bodies in comparison with the standard addition method, which was assumed as an accurate method not affected by matrix effects. Real samples of drinking water, surface water, and groundwater have been examined with this method in the Rhine and Ruhr region.

In 2007, two further polar pesticide metabolites, desphenyl-chloridazon (DPC) and methyl-desphenyl-chloridazon (MDPC) were reported with high potential to reach ground water bodies. High concentrations of DPC and MDPC were detected in many ground water and drinking water samples in Southern Germany and the region of Hesse. Also for DPC and MDPC, strong matrix effects were detected. In **Chapter 3** was firstly, a determination method for the polar metabolites DPC and MDPC in different water bodies developed. Secondly, a new approach to reduce matrix effects and to enhance ESI efficiency was investigated. The environmental matrix shows a strong ESI enhancement (matrix effect) on the DPC and MDPC signals, examined by the use of model samples that contain salts occurring in environmental samples (the

same as in Chapter 2). The strong effect of ESI enhancement was attributed to the ammonium ions. Therefore, the addition of ammonium ions was investigated with respect to enhancing the ESI intensity as well as to reducing the matrix effects when added in higher concentrations. A post-column infusion was applied and optimised for addition of ammonia solution (as source of ammonium ions) into the mobile phase – without changing the chromatographic separation of DPC and MDPC – to enhance the sensitivity and to reduce matrix effects. The chromatographic separation was optimised with regard to different chromatographic parameters (column phases, temperature, and gradient) including on-column focusing by LVDI UPLC-ESI-MS/MS for enhancing the signal to noise ratio. The remaining matrix effects were compensated by IS, of which one was self-synthesised. The reliability of this method was demonstrated in the same manner as described in Chapter 2 and real samples in the same region in Germany were examined.

In 2008, the Federal Office of Consumer Protection as well as Food Safety and the Federal Environment Agency published data about further polar pesticide metabolites (Table 1-1). As a result, sampling campaigns of individual pesticide metabolites showed the frequent occurrence of positive detections in ground water and drinking water in entire Germany. Therefore, in **Chapter 4** a reliable and economic routine multicomponent determination method for the pesticide metabolites in trace level was developed. Because lack of suitable IS for all metabolites, a standard addition method (SAM) was used to compensate occurring matrix effects. However, this method is time and labor intensive by manual handling. Therefore, this chapter describes a fully automated SAM for quantification of the 29 polar pesticide metabolites (Table 1-1) in different water bodies using UPLC-MS/MS. The automated SAM procedure carried out by a multi-purpose sample manager and a work flow for concentration and quality criteria calculation were developed. The method development included the optimisation of chromatography (reversed-phase material, temperature, gradient), multi-purpose sample manager (carry-over effects, repeatability) and mass spectrometer (enhancement of ESI intensity by post-column infusion of additives and desolvation temperature). The matrix effects and reliability of this method was investigated in different real water samples. Real samples of

different water bodies have been examined and evaluated for samples from the Rhine and Ruhr region of North Rhine-Westphalia.

As described in the introduction, it is known that the use of ozonation during water treatment can lead to the conversion of DMS to the carcinogenic NDMA. The behaviour during the chlorination process (safety or transport chlorination) of DMS was not completely examined, it was only known that the use of hypochlorite leads to degradation of DMS. Therefore, in **Chapter 5** exemplary investigations of DMS degradation using hypochlorite in accordance with chlorine disinfection were conducted. The chlorination was systematically investigated and formed transformation products were detected, identified, and characterised by analytical standards. Finally, the relevance of DMS transformation products for drinking water treatment was assessed.

1.4 References

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Chapter 2. Determination of the polar pesticide degradation product N,N-dimethylsulfamide in aqueous matrices by UPLC-ESI-MS/MS*

* *Redrafted from " Sebastian Kowal, Peter Balsaa, Friedrich Werres, Torsten C. Schmidt, Determination of the polar pesticide degradation product N,N-dimethylsulfamide in aqueous matrices by UPLC-MS/MS, Analytical Bioanalytical Chemistry, 2009, 395:1787 – 1794.*

2.1 Abstract

This study presents a fast, sensitive and robust method for the determination of the polar pesticide degradation product N,N-dimethylsulfamide (DMS) in water based on ultra-performance liquid chromatography/tandem mass spectrometry (UPLC-MS/MS). To provide a robust analysis method the use of an internal standard for both natural waters and model water was examined in order to compensate for matrix effects. The relative standard deviation was found to be +/-15% (n = 10) and the limit of detection was 10 ng/L by direct injection in the UPLC-MS/MS system. The only sample preparation step required is the addition of the internal standard. The chromatographic analysis of one sample takes four minutes and thus is applicable for economic routine laboratory work. More than six hundred samples of drinking water, surface water and ground water have been examined successfully with this method in the Rhine and Ruhr region of North Rhine Westphalia (Germany). Approximately 65% of analysed samples contained measurable amounts of DMS at concentrations up to 63 µg/L.

2.2 Introduction

Over the last years the production and application of pesticides has changed. Polar and more easily degradable pesticides replaced nonpolar and persistent ones [1]. Such polar pesticides tend to decompose to smaller molecules of increased polarity. Degradation products formed do not accumulate in soil and are often not removed effectively by state-of-the-art water treatment processes (e.g., activated carbon) [2]. In the past, such polar metabolites could hardly be detected with available analytical methods. Therefore, it took some time after the market launch of polar pesticides, until suitable instrumentation and analytical methods were available in order to detect such degradation products in the environment. The polar degradation product N,N-dimethylsulfamide (DMS) is formed by degradation of the pesticide tolylfluanid. Tolylfluanid has been used as fungicide in fruit culturing and wine culturing since approximately 1974. DMS was first detected in ground waters of Southern Germany in 2006. Found concentrations ranged between 0.1 and 1 µg/L [2]. Consequently, the outdoor application of tolylfluanid was banned in early 2007 [2]. Because of the high polarity (log D = -0.2 calculated by Sparc online calculator V4.2, 2008) DMS is not removed effectively by current water treatment processes such as bank filtration or activated carbon adsorption. Ozonisation converts DMS into the highly potent carcinogenic N-nitrosodimethylamine (NDMA) that is regulated at very low levels in drinking water (10 ng/L) [2]. Thus, ozonisation of DMS containing natural water is critical within a water treatment process even when DMS-concentrations in raw water are low. Therefore, a suitable analytical method for a routine monitoring of natural water and drinking water is necessary. Due to the polarity of DMS, conventional sample preparation methods such as solid-phase-extraction (SPE) and liquid-liquid-extraction (LLE) are not applicable. Therefore, direct injection of aqueous samples in a UPLC-ESI-MS/MS system is an attractive alternative. These instruments have been used for the measurement of polar pesticides for several years [3, 4]. However, usually the combination with SPE [3, 5, 6] is necessary to enable sufficiently low limits of detection to control the environmental quality standards given by the European Council Directive for drinking water (98/83/EC) [1, 7] (the concentration of any individual pesticide residue in drinking water is limited to 0.1 µg/L). The direct injection of aqueous samples that contain polar compounds was made possible by

the development of more sensitive analytical devices [3]. The application of ESI-MS/MS in combination with direct injection has one major drawback, because the measurement is affected significantly by the presence of other sample components (matrix), which lead to coelution and subsequently to either ion suppression or ion enhancement (so-called matrix effect) [8-13]. Consequently, the obtained results are inaccurate. It has been reported that the matrix has a strong influence on early eluting (k values up to 1 [14]) polar target compounds, whereas later eluting target compounds are little or not affected by the matrix [9, 12, 13, 15]. It has been suggested that the presence of both polar organic compounds (e.g. humic acids) and maybe inorganic compounds is responsible for matrix effects [6, 11, 14, 16, 17]. To ensure an accurate measurement of the target compounds, different methods are applicable in order to compensate for the matrix effects. A very simple method is the dilution of the sample [15, 18, 19]. However, the use of dilution is limited by the required limits of detection for the target compounds. An alternative method is the use of matrix-matched samples for calibration, which have a similar composition regarding the matrix components but do not contain the target compounds [11, 13, 14, 16, 19, 20]. Unfortunately, the composition of natural water samples varies in a broad range, so that the application of matrix-matched samples for calibration is not useful. A widely used method is standard addition, which provides very accurate results [4, 10, 15]. In practice, however, this method needs both a time-consuming sample preparation and evaluation of the obtained results and is therefore not suitable to establish a fast routine method [4, 19, 20]. Using a deuterium or ^{13}C-labeled internal standard is a fast and precise method, because the internal standard has nearly the same chemical properties as the target compounds and is influenced by the matrix effects in a similar way [4, 8, 14, 15, 18-20]. Thus, it is possible to ensure a reliable and automatic compensation of the matrix effects by using the instrument software. The sample preparation is easy, because only a certain amount of internal standard has to be added to the sample. The drawbacks of using the internal standard were that one IS would be required for each analyte and that stable isotope standards are not easily available for all compounds to be analysed.

To improve the limit of detection mobile phase additives such as formic acid, ammonium formate or ammonia were often used [4, 5, 9, 15, 21, 22]. Like the effects caused by matrix components, mobile phase additives can significantly influence the signal. They can lead to an enhancement or a reduction of the signal intensity [12]. In general, the influences of the additives depend on their concentration in the sample [4, 9, 10, 12]. Further advantages of additives are improvements of peak shape and reproducibility of retention times during the elution in UPLC [4, 5, 12, 22-24].

The aims of this study were (i) the development of a fast and robust analytical method using a deuterated internal standard and (ii) the investigation of different mobile phase additives and their influence on DMS response and other chromatographic parameters. After a successful optimisation and validation of the method, robustness was examined by measuring different natural and spiked model water samples. Therefore, the natural water samples were prepared both after the internal standard method and after the standard addition method. In the case of model water samples (examination of matrix effects), the robustness was determined by comparison of the values obtained by the internal standard method with an external calibration. Finally, sample preparation for routine analysis was simplified significantly by the internal standard procedure.

Many drinking water, surface water and ground water samples have been examined with this method in the Rhine and Ruhr region (Germany). The evaluated results are presented in this study in order to give an overview on concentrations of DMS in representative water bodies of North Rhine Westphalia.

2.3 Experimental

2.3.1 Reagents and materials

HPLC-MS/MS grade acetonitrile, water, trifluoroacetic acid and formic acid were obtained from Biosolve (Netherlands), N,N-dimethylsulfamide (DMS), purity 98%, was from ABCR GmbH & Co.KG (Germany). N,N-dimethylsulfamide (D_6) (in the following called D_6DMS), purity 99% and HPLC-MS/MS grade ammonium formate were purchased from Campro Scientific (Germany). Ultrapure water for dilutions was prepared by the water purification system Seralpur Pro 90 CN (Germany). Argon gas (5.0) for the collision cell was obtained from Air Liquide (Germany). Nitrogen for the mass spectrometric system was prepared by CMC instruments gas generator (Eschborn, Germany). Iron(II)chloride, iron(III)chloride, manganese(II)chloride, ammonium chloride, ammonium nitrate and ammonium sulfate were from Merck KGaA (Germany). All listed salts were analytical grade. Humic acid sodium salt (contains 45 – 60% (w/w) of humic acid) was from Carl Roth GmbH & Co.KG. A saturated solution of humic acid was prepared and the concentration of dissolved humic acid was analysed by DIN EN 1484. The dissolved organic carbon concentration of the humic acid solution was 3.0 mg/L and the total organic carbon concentration was 3.5 mg/L. Disposable nylon syringe filters, pore size diameter 0.2 µm, were from Pall Life Sciences (USA). 2-mL polypropylene syringes for the filters were obtained from Terumo (Slovakia). Glass syringes (100, 250, 500 µL) were from Hamilton Bonaduz AG (Switzerland).

2.3.2 Instrumentation and mass spectrometric conditions

All experiments were carried out on a Waters Acquity UPLC^{TM}-TQD ultra performance liquid chromatography coupled with an electrospray ionisation tandem mass spectrometric system (Milford, USA). The following UPLC columns were investigated for separations: BEH C_{18} 2.1 x 50 mm, 2.1 x 100 mm, BEH C_8 2.1 x 50 mm, particle size of both types were 1.7 µm and HSS T3 2.1 x 100 mm, 1.8 µm particle size (Waters, Milford, USA). The mass spectrometric operating parameters were optimised in full scan mode (m/z 40 - 200) using an infusion system

with a mixing tee. The DMS solution (10 µg/L) was infused with a flow rate of 10 µL/min using one line of the mixing tee, and 0.1% formic acid in acetonitrile/water (2:98, v/v, mobile phase) was added at 350 µL/min using the other line. The final ESI-MS conditions were as follows: the tandem mass spectrometer was used in the positive ESI mode; drying gas N_2 (1000 L/h, 450°C); capillary voltage 800 V; cone voltage 27 V. Collision gas was argon at a flow rate of 0.15 mL/min. The collision energy was adapted on the selected ions. The $[M + H]^+$ ions were used in this study as precursor ions for the MS/MS experiments. The m/z values of the precursor ions, product ions, and the collision-induced dissociation (CID) energy for the quantification transitions in the selected reaction monitoring (SRM) mode are listed in Table 2-1.

Table 2-1: Settings of tandem quadruple for the precursor–product transitions. (*) Transitions used for quantification.

Name	M	Q1 m/z $[M+H]^+$	Q2 m/z $[M+H]^+$	CID energy (eV)	dwell time (s)
DMS	124	124.8	107.8*	11	0.05
		124.8	44.3	17	0.02
D_6DMS	130	130.9	114.0	16	0.02
		130.9	51.0*	16	0.05

2.3.3 Examination of mobile phase additives

The effect of varying additive concentrations on the ionisation process of DMS was studied by using solutions of formic acid and ammonium formate infused with a mixing tee. Successively increasing concentrations of additives were added to the mobile phase (0, 0.01, 0.025, 0.05, 0.1% v/v formic acid or ammonium formate with a concentration of 1 mol/L). The DMS solution of 10 µg/L in pure water was infused with a flow rate of 10 µL/min into the mobile phase (flow rate 350 µL/min) with different concentrations of additives. The response of the DMS product ion (m/z 107.8) was continuously monitored.

2.3.4 Chromatographic conditions

An acetonitrile/water mixture (2:98, v/v, mobile phase) was used as mobile phase with a constant flow rate of 360 µL/min. Several column types and lengths were used (as described above). The columns were operated at 15, 30 and 45°C. Each parameter combination was run 10 times. The conditions with the lowest standard deviation and highest signal intensity were selected for further measurements. Pure water, 0.01% v/v formic acid and 0.5 mmol/L ammonium formate solutions were compared as aqueous mobile phase components. For the final separation conditions, a Waters UPLC HSS T3 C18 column (2.1 x 100 mm, 1.8 µm particle size) equipped with a sinter layered filter plate (2 µm), and a column oven temperature of 15°C were used. Samples (50 µL each) were injected by an auto sampler. The mobile phase consisted of acetonitrile/0.01% v/v formic acid. For the separation of DMS, the ratio of acetonitrile/formic acid was kept at 2:98 for the first 2.3 min (isocratic elution). Afterwards the content of acetonitrile was increased linearly up to 98% within 0.2 min and this ratio was kept constant for 0.3 min (cleaning phase). In a final step, starting conditions were re-established within 0.2 min and maintained for 0.8 min (conditioning phase). The flow rate was 360 µL/min and the total runtime was 4 min. The measurement of dead time was carried out with trifluoroacetic acid, because it does not interact much with reversed phase columns. Due to its strong negative inductive effect, trifluoroacetic acid causes ion suppression, and thus is easy to observe in the chromatogram [4, 18].

2.3.5 Instrumental conditions for routine measurement

For the measurement of real water samples, the following procedure was applied: First, five blank pure water samples were analysed in order to condition the column. Afterwards, reference solutions (preparation is described in the following paragraph) were measured, followed by measurement of real water sample. To minimise the contamination of the instrument with non-volatile sample matrix a valve switching mechanism was introduced that controlled the flow of the mobile phase to the detector. Only within a short time (1.4 min), when DMS was eluted, the mobile phase was directed to the detector. All measurements for both validation and quantification of real samples were conducted with the final spectrometric and chromatographic

conditions of the instrument. Peak areas were processed and integrated automatically. Any occurring matrix effect was compensated by internal standard procedure.

2.3.6 Sample preparation and reference solutions

Stock solutions of DMS and D_6DMS were prepared by weighing and dissolving in acetonitrile. The stock solutions had a DMS and D_6DMS concentration of 100 mg/L. They were stored at 4°C and were used for the preparation of diluted reference solutions (see reference solutions for calibration). For spiking the water and model samples, spike solutions diluted by pure water with concentrations of 0.1, 0.01 µg/L DMS and 0.1 µg/L D_6DMS were used. Final spiked water samples and standard solutions contained less than 0.1% of acetonitrile.

2.3.7 Reference solutions for calibration

Aqueous standard solutions were prepared by diluting the DMS stock solutions with pure water to yield a concentration range of 0.03 – 0.3 µg/L and 0.3 – 3.3 µg/L, respectively. The first range comprised seven calibration solutions with final concentrations of 0.03, 0.05, 0.08, 0.13, 0.18, 0.28 and 0.33 µg/L. The second range comprised six calibration solutions with final concentrations of 0.33, 0.5, 1.5, 2.1, 2.7 and 3.3 µg/L. All standard solutions contained 0.5 µg/L D_6DMS. The solutions were used to check the linearity of the method. Two DMS solutions (0.05 and 1.5 µg/L) were measured ten times in order to determine the relative standard deviation.

2.3.8 Ruggedness testing

Model solutions of different salts and humic acid were prepared by weighing and dissolving in 100 mL pure water. The following final concentrations were used for examination of occurring matrix effects: ammonium chloride (60 mg/L), ammonium nitrate (100 mg/L), ammonium sulfate (120 mg/L), humic acid (3 mg/L), a mix of iron(II/III)chloride (7 mg/L) and manganese(II)chloride (1.5 mg/L). To the prepared model samples and one pure water blank, DMS and D_6DMS spike solutions were added to yield final concentrations of 2 µg/L and 0.5 µg/L, respectively. Each of the

model water samples was measured five times with the final instrumental conditions. In order to determine the robustness of the method, real water samples (10 ground water, 5 surface water, and 5 drinking water) were prepared as follows. Each sample was divided in 4 aliquots. The aliquots were spiked with different DMS concentrations (0, 0.2, 0.4, 0.8 µg/L). Afterwards, the internal standard was added to each of the aliquots with a final concentration of 0.5 µg/L D_6DMS. The quantification for standard addition was obtained by linear regression. The use of internal standard was automatically calculated.

2.3.9 Sample preparation for routine measurements

All real water samples to be analysed (ground water, surface water and drinking water) were transferred into 100-mL flasks (Duran glass). Afterwards, the sample amounts in the flasks were determined by weighing and, if necessary, the water samples were filtered. Finally, 100 µL of the D_6DMS spiked solution was added to the water samples by a glass syringe to obtain a D_6DMS concentration of 0.5 µg/L in the water sample.

2.4 Results and discussion

2.4.1 Optimisation of UPLC-MS/MS conditions

As reported in [12-15], matrix influences of early eluting polar target compounds was very strong. To achieve a good separation from the sample matrix, the analyte should remain as long as possible on the column [13, 18]. To give adequate resolution in a reasonable analysis time, retention factors k between 1 and 10 are required [26]. Using an acetonitrile/0.01% v/v formic acid ratio of 2:98% (v/v) a k value of 2.2 for DMS was determined. For the highest sensitivity in quantitative analysis by UPLC-MS/MS, the most intense product ion of DMS was selected for quantification purposes. However, the background noise level of the most intense transition of D_6DMS (m/z 130.9 > 114, CID 16 eV) was relatively high under the present conditions. This interference is due to the mobile phase and influence especially the background noise of the mass transition 130.9 to 114 of the internal standard D_6DMS whereas the transition of mass 130.9 to 51.00 was not affected. Therefore, this transition was selected for quantification. The ESI-MS/MS spectra and the proposed fragmentation reactions of protonated DMS are shown in Figure 2-1. The other MS/MS detection conditions (ion source voltage, CID and flow rate of nitrogen gas) were also optimised to obtain the highest signal intensities. Under the described UPLC-MS/MS conditions, the complete chromatographic run took four minutes.

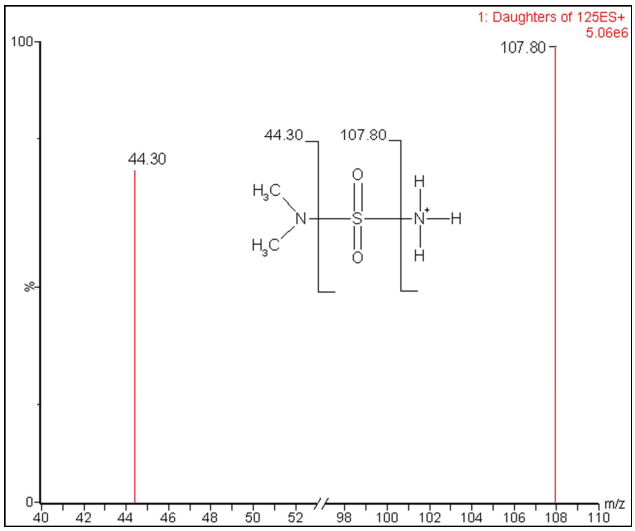

Figure 2-1: Product ions produced by the precursor DMS (m/z 125 for $[M+H]^+$).

2.4.2 Influence of different mobile phase additives on signal response

It is described in the literature [4, 5, 9, 12, 15, 22] that different additives such as formic acid or ammonium formate in the mobile phase are known to increase the MS response of analytes, because they are good proton donors or acceptors during the ionisation process [9]. The maximum response for DMS was obtained either with formic acid at 0.01% v/v or with ammonium formate at a concentration of 0.5 mmol/L as shown in Figure 2-2. The enhancement of the response of DMS is factor 2.5 for formic acid and factor 2.2 for ammonium formate. Furthermore, the presence of such additives in the mobile phase has been reported to improve reproducibility of analyte retention time and peak shape for compounds with ionisable groups [4, 12]. In contrast, for DMS chromatographic performance was not enhanced by using either of the compounds. This is related to the two extreme pK_a values of DMS (-8.55 and 18.03; Sparc online calculator V4.2, 2008). In the interesting pH-range of 2 - 3 (mobile phase with formic acid 0.01%) DMS exists only as a neutral molecule and shows none acid-base behaviour. Finally, formic acid at 0.01% v/v was chosen as a mobile phase additive because in addition to signal enhancement the possible inorganic scaling on the surfaces of the ion source was reduced.

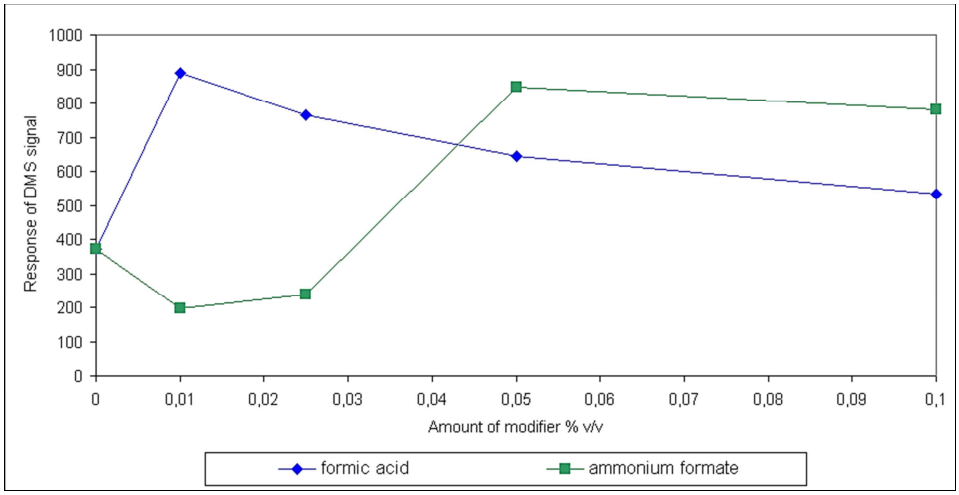

Figure 2-2: Influence of different mobile phase additives on the response of the DMS signal.

2.4.3 Validation

For the quantitative analysis of DMS, two calibration ranges were used for a better linearity over two orders of magnitude. The first calibration range was from 0.03 to

0.33 µg/L and the second from 0.33 to 3.3 µg/L. The relative standard deviation (%RSD) was calculated from a sample containing 0.05 µg/L and 1.5 µg/L of DMS, respectively (n = 10). The limit of quantification (LOQ) was defined as a signal-to-noise (S/N) ratio of 10:1 and the limit of detection (LOD) as a S/N ratio of 3:1 (see Figure 2-3). The chosen (S/N) ratios of LOQ and LOD are typical values for this kind of application [3, 17, 20]. The summarised validation data are as follows: For the concentration range from 0.03 - 0.33 µg/L a %RSD of 15% and a coefficient of correlation (R^2) of 0.991 were obtained. For the concentration range from 0.33 - 3.3 µg/L the values of %RSD and R^2 were slightly better, with 7% and 0.996, respectively. The LOQ was 0.03 µg/L and LOD 0.01 µg/L.

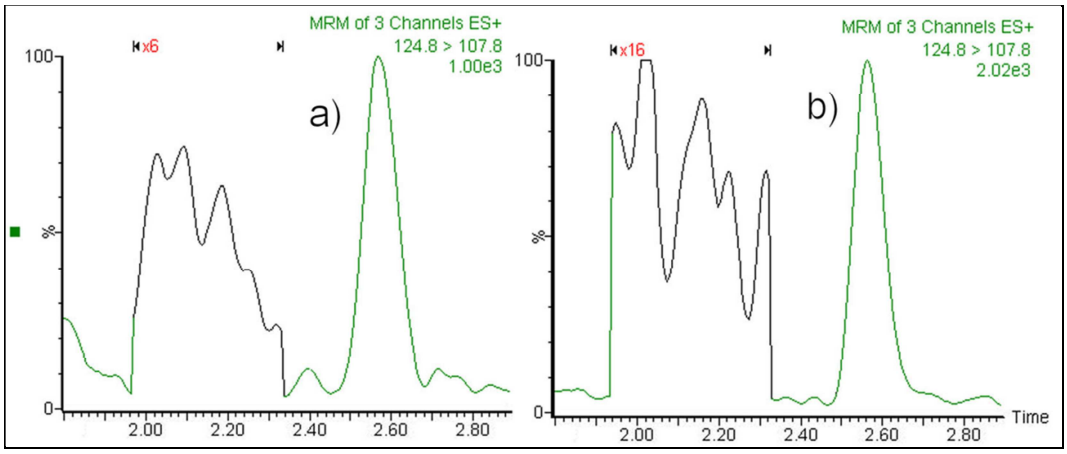

Figure 2-3: DMS peak at 2.58 min obtained by measuring drinking water containing 0.01 µg/L DMS (a) and well water with 0.03 µg/L DMS (b). Noise was zoomed 6 x and 16 x, resulting in S/N 3:1 (LOD) and 10:1 (LOQ).

2.4.4 Matrix effects in model waters

To investigate potential matrix effects in environmental water samples that may have a significant influence on the DMS signal, different model waters with various inorganic salts and spiked humic acid were used. Previous to these experiments, data of 600 environmental samples (ground water, surface water and drinking water) were evaluated with regard to the most frequent matrix substances and their concentrations. The 95%-quantile value of the concentrations was calculated to exclude outliers, and these concentrations were used for further experiments. The concentration and variety of model waters used in this work is described in the experimental section. Figure 2-4 shows the influences of different matrix constituents

on DMS response. These influences are compensated well by using the internal standard. Chloride and nitrate anions have a negative influence on the quantification of DMS, because they decrease the DMS signal, whereas the other matrix constituents do not affect the DMS signal. One reason for the negative influence of chloride and nitrate is maybe their higher concentration compared with iron or manganese cations or humic acid. Another possible reason is that in electrospray, analytes are protonated in the liquid phase inside electrically charged droplets. Chloride and nitrate act as strong proton acceptors in the ion source, therefore may interfere with the ionisations process. Unfortunately, the understanding of the ESI-MS mechanism is not yet conclusive, thus ion suppression by anions warrants further investigation [22, 27].

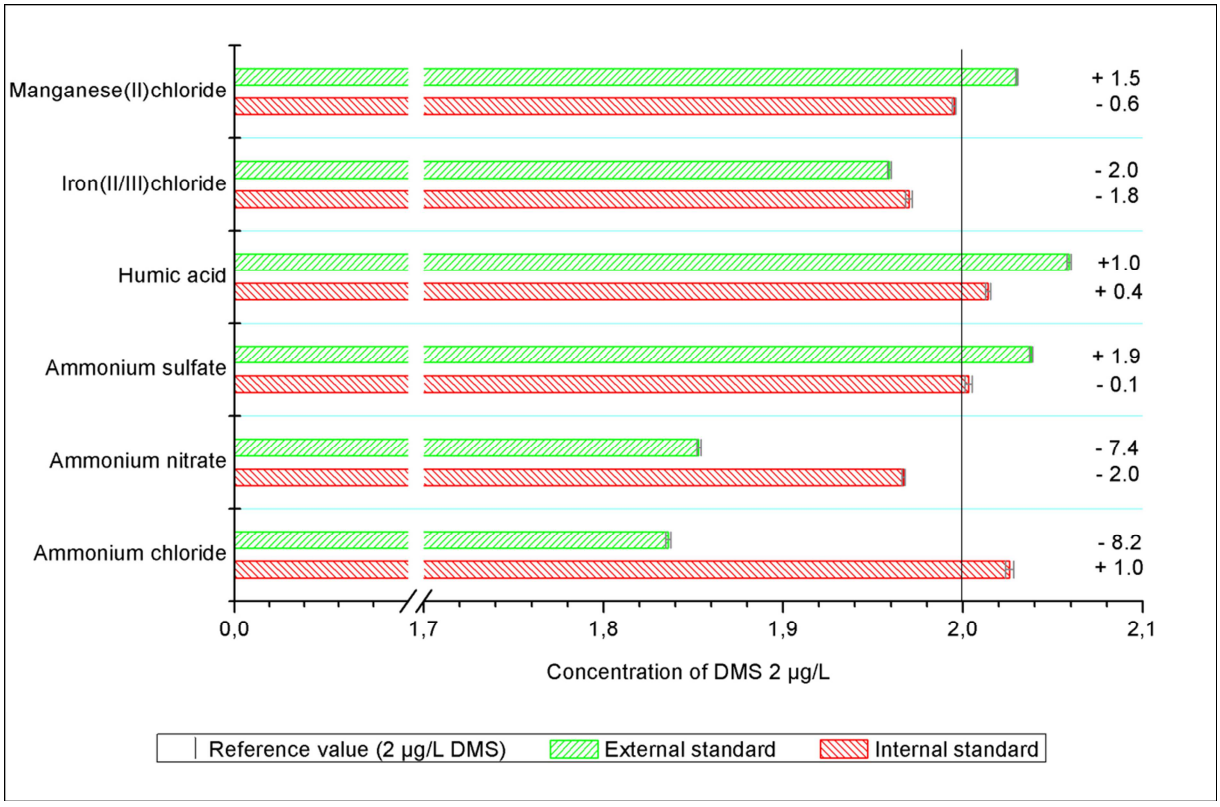

Figure 2-4: Comparison of the compensation of matrix effects by internal and external standard procedures for different model water samples. DMS concentration in each sample was 2 µg/L. The vertical line on the right side marks the DMS references value of 2 µg/L in pure water. The values on the right side show the deviation (%) of DMS concentrations in model water samples compared with the DMS reference value in pure water.

2.4.5 Method robustness and suitability for routine analysis

To corroborate the robustness of the internal standard compensation of matrix effects in real samples, ten ground water, five surface water and five drinking water samples were analysed and the results compared to those obtained by standard addition method. The assumption was that standard addition method provides the most accurate value of the analyte concentration and is the most effective albeit laborious way to eliminate any matrix effect [10, 16, 19]. Figure 2-5 shows that the concentrations quantified by the use of D_6DMS as internal standard are comparable with those obtained by standard addition method. DMS values obtained by the internal standard method have deviations of only 5% compared to DMS values measured by standard addition method. These results demonstrate the robustness of internal standard calibration for any remaining matrix effects in the environmental water samples.

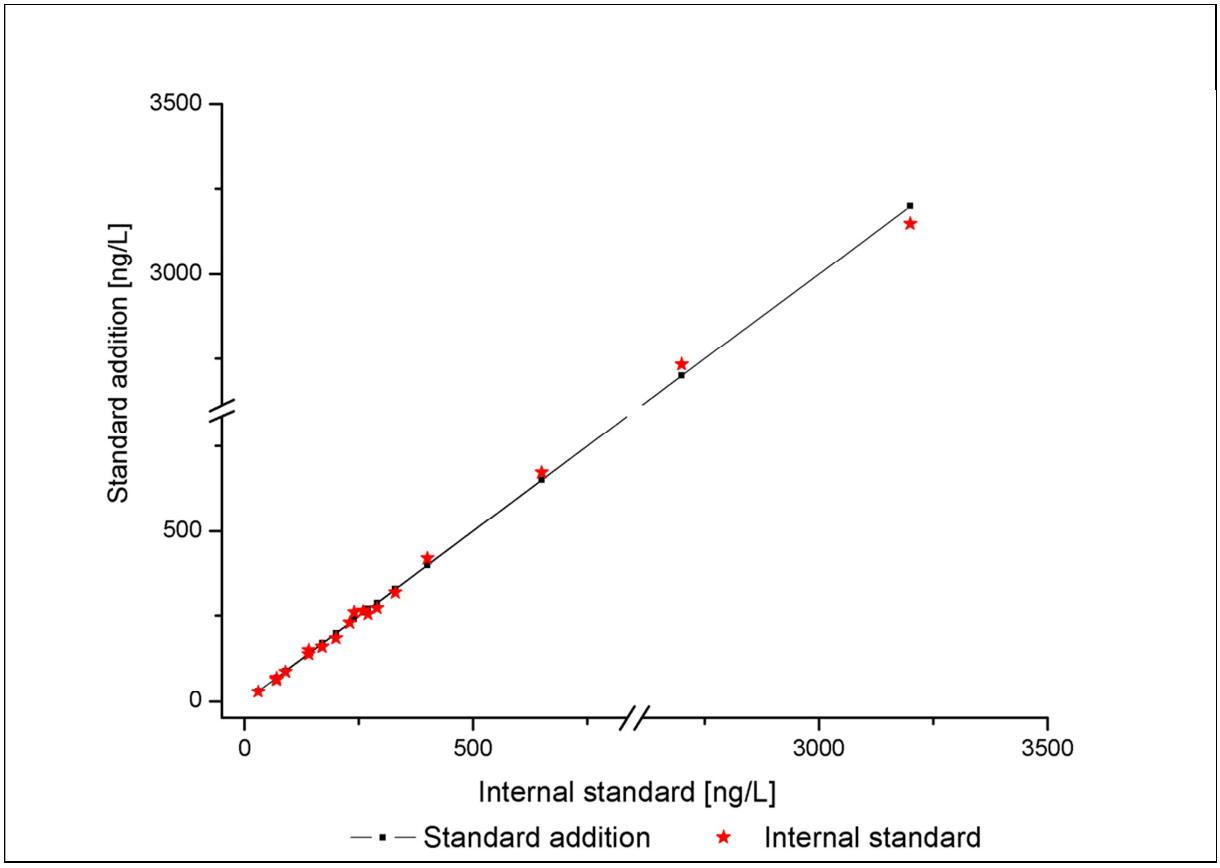

Figure 2-5: Correlation of DMS concentrations determined in 20 real samples by the internal standard method and the standard addition method.

About 600 drinking water, surface water, and groundwater samples were analysed by the described method for DMS. Figure 2-6 summaries the statistical data of the different water bodies in box plots. Approximately 65% of all samples contained DMS, 46% of samples showed DMS concentrations of more than 0.10 µg/L. Few ground water samples contained higher concentrations (up to 63 µg/L) of DMS. Only 30% of analysed groundwater samples showed a concentration below 0.1 µg/L. The 90-percentile value was 6.1 µg/L. The DMS concentration in water samples from three different water treatments plants before and after the treatment with active carbon was quantified. It could be shown that the removal of DMS by active carbon adsorption was not effective, corroborating previously reported results [2].

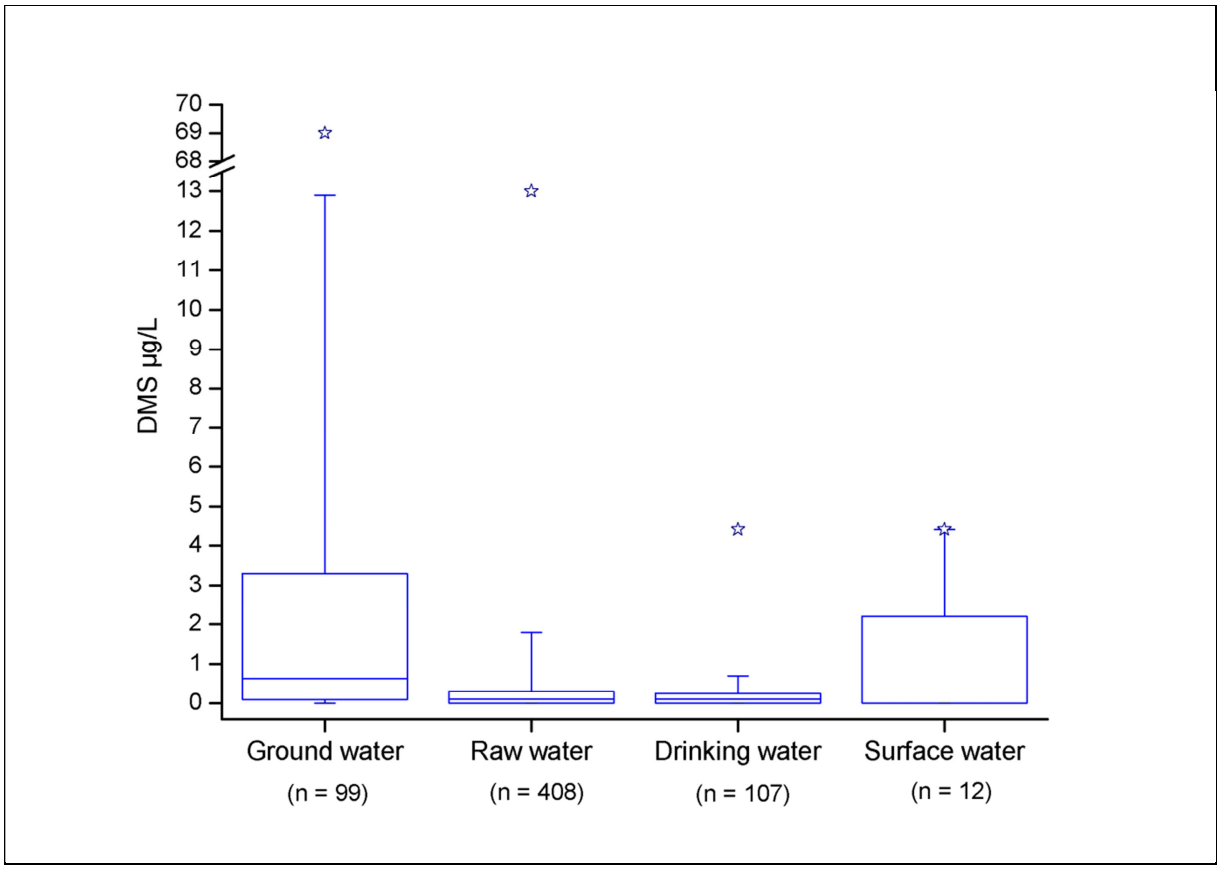

Figure 2-6: Box-whisker plots showing DMS concentrations in various environmental water samples (ground water, raw water, drinking water and surface water). The line in boxes represents the median, the boxes the 25 - 75% percentile, the whisker extends to the 95%-percentile of measured DMS concentrations. The stars indicate the highest DMS concentrations.

2.5 Conclusions

A robust, fast and sensitive analysis method for the detection of the pesticide metabolite DMS was developed for quantification in ground water, surface water, and drinking water. This method fulfils the requirements of the 0.1 µg/L limit set by the European Council Directive for drinking water (98/83/EC) and allows for cost-efficient analysis. With formic acid as mobile phase additive, the response of the DMS signal was enhanced 2.5 times. The robustness of this method was analysed with model waters and real water samples. As a result, despite ion suppression caused by matrix from the water samples, the possible deviations for DMS were compensated satisfactory by the deuterated internal standard.

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Chapter 3. Reduction of matrix effects and improvement of sensitivity during determination of two chloridazon degradation products in aqueous matrices by using UPLC-ESI-MS/MS*

** Redrafted from " Sebastian Kowal, Peter Balsaa, Friedrich Werres, Torsten C. Schmidt, Reduction of matrix effects and improvement of sensitivity during determination of two chloridazon degradation products in aqueous matrices by using UPLC-ESI-MS/MS, Analytical Bioanalytical Chemistry, 2012, 403:1707 – 1717.*

3.1 Abstract

The development and validation of a sensitive and reliable detection method for the determination of two polar degradation products, desphenyl-chloridazon (DPC) and methyl-desphenyl-chloridazon (MDPC) in surface water, ground water and drinking water is presented. The method is based on direct large volume injection UPLC-ESI-MS/MS (ultra performance liquid chromatography electrospray tandem mass spectrometry). This simple but powerful analytical method for polar substances in the aquatic environment is usually hampered by varying matrix effects, depending on the nature of different water bodies. For the two examined degradation products, the matrix effects are particularly strong compared to other polar degradation products of pesticides. Therefore, matrix effects were studied thoroughly with the aim to minimize them and to improve sensitivity during determination by post-column adding of ammonia solution as a additive. An internal standard was used in order to compensate remaining matrix effects. The calibration curve shows very good coefficients of correlation (0.9994 for DPC and 0.9999 for MDPC). Intraday precision values were lower than 5% for DPC, 3% for MDPC and the limits of detection were 10 ng/L for both substances. The method was successfully used in a national round robin test with a deviation between 3 - 8% from target values. Finally, about 1000 samples from different water bodies have been examined with this method in the Rhine and Ruhr region of North Rhine-Westphalia (Germany) and in the European Union. Approximately 76% of analysed samples contained measurable amounts of DPC at concentrations up to 8 µg/L, while 53% of the samples showed MDPC concentrations up to 2.3 µg/L.

3.2 Introduction

Within a monitoring programme held by the Bavarian Environment Agency (Germany) in 2006, the occurrence of different plant protection products in ground water and surface water was investigated, including among others the pesticide chloridazon and its degradation product desphenyl-chloridazon (DPC) known from lysimeter tests [1]. High concentrations of DPC up to 10 µg/L were found in many ground water samples. At about the same time, another hitherto unknown degradation product, methyl-desphenyl-chloridazon (MDPC), was detected and characterised in water samples from Baden-Wurttemberg [2]. Subsequent sampling campaigns in Southern Germany and the region of Hesse have confirmed high frequency of positive detections for DPC and MDPC in ground water and drinking water, whereas low concentrations of the parent substance, chloridazon, were determined [3]. Pan-European surveys show also frequent occurrence of the two degradation substances [4]. Both substances are stable degradation products of the herbicide chloridazon, which has been used since 1964 for sugar beet, beetroot and onion cultivation [3]. In 2007, approximately 25 - 100 t/a of chloridazon were sold in Germany. The microbial degradation of chloridazon in fields produces DPC and MDPC within six to eight weeks [5]. Both degradation products (show in Figure 3-1) are very polar substances with estimated log P values of -0.84 and -0.62, respectively [6], which indicates rapid transport throughout the unsaturated zone and within an aquifer due to negligible sorption. Furthermore, the high polarity of DPC and MDPC prevents effective removal by current water treatment processes such as bank filtration or activated carbon adsorption. The frequent occurrence and rather high concentrations (> 1 µg/L) in groundwater and drinking water require an intensive surveillance of both degradation products.

Standard sample preparation methods such as Solid-Phase-Extraction (SPE) or Liquid-Liquid-Extraction (LLE) are time consuming and not effective though, because the high polarity of DPC or MDPC causes low recoveries [2, 3]. For highly polar and low volatile substances liquid chromatography mass spectrometry (LC-MS) is the adequate detection method [7]. In a previous study it was shown, that for a very polar pesticide degradation product, N,N-dimethylsulfamide (DMS), direct large volume

injection and application of an internal standard in connection with electrospray ionisation tandem mass spectrometry (ESI-MS/MS) fulfilled the requirements for a reliable quantification of such substances on the trace level [8]. However, many water constituents such as inorganic salts or humic substances affect the ionisation efficiency during LC-ESI-MS/MS detection [9]. The presence of such matrix components frequently leads to an increase or decrease of response intensity, the so-called matrix effect [9-14]. For DPC and MDPC, this effect proved to be particularly relevant. Furthermore, a national round robin test including 38 laboratories demonstrated that the precise quantification of DPC and MDPC is difficult [15]. Therefore, it was necessary to develop a reliable analytical method for the detection of both degradation products in various water samples and to validate the robustness of this method. Consequently, the direct large volume injection was used in connection with LC-ESI-MS/MS; subsequent optimisation measures and robustness tests were conducted.

Goals of this work was (i) to eliminate the disadvantages of direct large volume injection such as tailing and to reach a higher retention on the chromatographic column for both substances to separate them from early eluting salts, which led to significant matrix effects; (ii) to investigate the matrix effect by artificial samples, which contained matrix components frequently found in environmental samples; (iii) to find ways to overcome this matrix effect, thereby increasing sensitivity and robustness of the analytical method; and (iv) to test the potential of isotopically labeled internal standards (commercial $^{15}N_2$-DPC and self-synthesised $^{15}N_2$-MDPC) to compensate matrix effects as much as possible. After these validation and verification procedures, the robustness was examined by comparison of the final analytical method with a standard addition procedure during the processing of environmental samples.

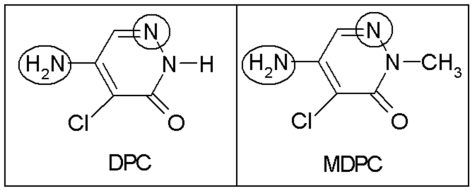

Figure 3-1: Molecular formulas of the DPC and MDPC with functional groups that are involved in the ionisation process.

3.3 Experimental

3.3.1 Reagents and materials

LC-MS grade acetonitrile, water, trifluoroacetic acid and formic acid were obtained from Biosolve (Netherlands). Desphenyl-chloridazon (DPC) and methyl-desphenyl-chloridazon (MDPC) solved in acetonitrile (100 mg/L) with a purity of 99% were purchased from Dr. Ehrenstorfer GmbH (Germany). Isotopically labeled desphenyl-chloridazon ($^{15}N_2$-DPC) was acquired from Campro Scientific GmbH (Germany) and ammonia solution (25%, analysis grade) from Merck KGaA (Germany). Ultrapure water for dilutions was prepared by the water purification system Seralpur Pro 90 CN from Seral (Germany). For the synthesis of internal standard N-Methyl-N-nitroso-p-toluenesulfonamide (Diazald®), purity 99%, was obtained from Sigma-Aldrich (Germany) and diethyleneglycol, analysis grade, from Merck KGaA. Ethyl acetate, diethyl ether and tetrahydrofuran for residue analysis were purchased from LGC-Standards GmbH (Germany). Sodium hydroxide and acetic acid were from Merck KGaA. Argon gas (5.0) for the collision cell was obtained from Air Liquide (Germany). Nitrogen for the mass spectrometric system was prepared by CMC instruments gas generator (Eschborn, Germany). Iron(II)chloride, iron(III)chloride, manganese(II)chloride, ammonium chloride, ammonium nitrate and ammonium sulphate were from Merck KGaA. All listed salts were analytical grade. Humic acid sodium salt (contains 45 – 60% (w/w) of humic acid) was from Carl Roth GmbH & Co.KG. A saturated solution of humic acid was prepared and the concentration of dissolved humic acid was analysed by DIN EN 1484 [16]. The dissolved organic carbon concentration of the humic acid solution was 3.0 mg/L and the total organic carbon concentration was 3.5 mg/L. Glass syringes (100, 250, 500 µL) were from Hamilton Bonaduz AG (Switzerland).

3.3.2 Instrumentation and mass spectrometric conditions

Waters Acquity UPLC™-TQD ultra performance liquid chromatography coupled with an electrospray ionisation tandem mass spectrometric system (Milford, USA) was used for all experiments. The following reversed phase UPLC columns were

investigated for separations: BEH C_8 2.1 x 50 mm and BEH ShieldRP18 C_{18} 2.1 x 50 mm, particle size of both types was 1.7 µm. All columns were equipped with a 0.2 µm sinter layered in-line filter, also purchased from Waters. As mobile phase acetonitrile and water with a constant flow rate of 360 µL/min was used (see below). The final ESI-MS/MS conditions were: ESI positive mode, drying gas N_2 (1000 L/h, 480°C); capillary voltage 0.3 kV and collision gas argon at a flow rate of 0.15 mL/min. The m/z values of the precursor ions, product ions, cone energy and the collision induced dissociation (CID) energy for the quantified transitions used in the selected reaction monitoring (SRM) mode are listed in Table 3-1. The optimisation of the chromatography conditions comprised two column types and several gradients for the mobile phase. Each parameter combination was run 10 times. The conditions with the lowest standard deviation and highest signal intensity as well as best peak shape were selected for further measurements. In order to optimize the ionisation process for both substances, different concentrations of formic acid (0.0, 1.3, 2.7, 4.0, 5.3, 6.6 mmol/L) as mobile phase additive in pure water and acetonitrile were investigated. For the final separation conditions, the UPLC BEH ShieldRP18 (C_{18} 2.1 x 50 mm, 1.7 µm) column with a column oven temperature of 40°C was used. Samples (50 µL each) were injected by an autosampler and the mobile phase composition was water/acetonitrile, both with 0.01% v/v formic acid. The mobile phase conditions are described in Table 3-2. The total runtime was 4 min. In this context, a solvent divert valve placed between the LC column outlet and the mass spectrometer inlet is used to save the ionisation source from both early and late eluting matrix components (such as inorganic salts or late eluting organic substances). During the elution of the two analytes the valve was switched in the period from 0.5 to 1.5 minutes to the MS, otherwise, the position was on waste.

The measurement of dead time was carried out with trifluoroacetic acid, because it does not interact much with any reversed phase adsorbent used in columns. Due to its strong negative inductive effect, trifluoroacetic acid causes ion suppression in electrospray ionisation and thus is easy to observe in the chromatogram [7, 17]. Examination of mobile-phase modifiers that have shown an enhancement of the ionisation process for DPC and MDPC (see section below), was conducted by using solutions of different ammonia concentrations infused via a mixing tee downstream

the chromatographic column (so-called post-column infusion). During the continuous monitoring of both substances, various ammonia solution concentrations (0, 5, 10, 21, 31, 41 mmol/L) were introduced with a constant flow rate of 15 µL/min through the mixing tee into the mobile phase with a flow rate of 360 µL/min (concentrations of ammonia in total flow were: 0.00, 0.14, 0.29, 0.58, 0.86 and 1.15 mmol/L). Thereafter, various flow rates (5 µL/min to 35 µL/min in steps of 5 µL/min) of ammonia solution were investigated with the previously identified optimum concentration (10 mmol/L) of ammonia. This investigation was also repeated with ultrapure water without ammonia.

Table 3-1: Mass-to-charge ratios (m/z) of the precursor ions, product ions, cone voltages and the collision induced dissociation (CID) energies used (* mean selected quantifier mass).

m/z of precursor ion	m/z of product ion	dwell time in sec	cone voltage (V)	CID energies (eV)
Desphenyl-chloridazon and $^{15}N_2$-desphenyl-chloridazon				
145.93	54.01	0.05	47	21
145.93	117.00*	0.05	47	20
149.9	103.96	0.05	50	20
149.9	118.92*	0.05	50	20
Methyl-desphenyl-chloridazon and $^{15}N_2$-methyl-desphenyl-chloridazon				
159.96	88.00*	0.05	44	30
161.97	88.00	0.05	44	30
163.98	89.79*	0.05	45	28
163.98	118.86	0.05	45	23

Table 3-2: Chromatographic conditions (gradient table).

Time (min)	Flow rate (mL/min)	% A	% B	curve
0	0.36	99.9	0.1	1
0.2	0.36	90	10	1
2	0.36	90	10	6
2.2	0.36	2	98	6
2.5	0.36	2	98	6
2.7	0.36	99.9	0.1	6
3.3	0.36	99.9	0.1	6

3.3.3 Synthesis of internal standard

For the synthesis of isotopically labeled $^{15}N_2$-MDPC as internal standard, the following solvents were investigated: ethyl acetate, diethyl ether and tetrahydrofuran.

First, 1 mL of isotopically labeled stock solution of $^{15}N_2$-DPC (94 mg/L) was evaporated in a test tube with nitrogen to dryness and resolved in 1 mL of one of the three solvents. These solutions were exposed for 30 seconds to diazomethane at -10°C (ice/NaCl mixture in the ratio of 1:2) and after 10 minutes evaporated with nitrogen up to dryness. Then, the dry solid was dissolved with 1 mL acetonitrile and used as stock solution. Diazomethane was prepared as follows: A solution of diethyl ether/diethylene glycol 1:1 (v/v) and 40% w/v KOH solution (5 mL each) were placed into a gas-tight test-tube, a spatula Diazald ® was added to this solution and the formed diazomethane was immediately introduced into the solvent containing the isotopically labeled $^{15}N_2$-DPC via a glass capillary. The yield of the synthesis was quantified with the method described above. The required mass transitions and instrument settings were taken from the isotopically labeled $^{15}N_2$-DPC and adapted to the mass of the isotopically labeled $^{15}N_2$-MDPC. For the quantification of the isotopically labeled $^{15}N_2$-MDPC yield, 50 µL of the solution were diluted with ultrapure water to the final concentration of about 10 µg/L (assuming 100% yield) and quantitated. The obtained response of isotopically labeled $^{15}N_2$-DPC was compared with synthesised isotopically labeled $^{15}N_2$-MDPC. A nearly 100% yield was achieved for methylation only in diethyl ether, while in ethyl acetate and tetrahydrofuran only 50% and 70% conversion took place, respectively, which is consistent with literature [18]. Therefore, diethyl ether was used as solvent for the synthesis of the internal standard.

3.3.4 Sample preparation, reference solutions, validation and quantification

The stock solutions were stored at 4°C and used for the preparation of diluted reference or spike solutions. Aqueous reference solutions for the external calibration were prepared by diluting the stock solutions with pure water to yield a concentration range of 0.01 – 1 µg/L with nine calibration points. All calibration solutions contained 0.5 µg/L internal standards. These solutions were also used to check the linearity, limit of quantification (LOQ), limit of detection (LOD) and two solutions (0.1 and 1 µg/L) were measured ten times in order to determine the relative standard deviation. Standard addition solutions were also prepared by diluting the stock solutions with ultrapure water to a final concentration of 0, 100, 200 and 300 ng/L.

These solutions were used for the quantification of the degradation products in real water samples by the procedure described below. Four aliquots (each 500 µL) of real sample were mixed with the standard addition solutions using four different vials.

3.3.5 Influence of matrix components

The occurring of matrix effects was investigated by artificial solutions containing humic acid and salts often occurring in the aquatic environment. The solutions were prepared by weighing and dissolving the solids in 100 mL pure water. The examined salts and concentrations are listed in Table 3-3. To the prepared artificial samples and a pure water blank, both degradation products (finally concentration: 1 µg/L) and isotopically labeled standards spike solutions (0.5 µg/L) were added. Each of the artificial water samples was measured at the final instrumental conditions.

Table 3-3: The 95%-quantile values of major ion concentrations of more than 600 environmental samples (ground water, surface water, and drinking water) that were evaluated with regard to the most frequent matrix substances and their concentrations.

	chloride	nitrate	sulphate	DOC	iron (II/III)	magnesium (II)
95-quantile value [mg/L]	59	107	121	3.8	6.8	17
95-quantile value [mmol/L]	1.69	1.73	1.26	-	0.12	0.69

3.3.6 Robustness testing

In order to determine the robustness of the analytical method, results derived from processing real water samples (different ground water, surface water and drinking water) according to two quantification methods were compared, on the one hand external calibration with correction by internal standards, on the other hand standard addition method (see sample preparation chapter above).

3.3.7 Sample preparation for routine measurements

All real water samples to be analysed (ground water, surface water and drinking water) were transferred into 100-mL flasks (Duran glass). Afterwards, the sample

volumes in the flasks were determined by weighing. Finally, 100 µL of the isotopically labeled spiked solution (500 µg/L) was added to the water samples by a glass syringe to obtain a concentration of 0.5 µg/L in the water sample. For the quantification of both degradation products, external calibration with internal standard correction and the above-mentioned chromatographic and spectrometric instrumental conditions were used.

3.4 Results and discussion

3.4.1 Optimisation of UPLC-ESI-MS/MS conditions

For the highest sensitivity in quantitative analysis by UPLC-ESI-MS/MS, the most intense product ion of DPC and MDPC was selected for quantification purposes. The isotopically labeled internal standards contain two ^{15}N-atoms in the ring structure, resulting in a mass difference of two amu. However, both DPC and MDPC contain a chlorine atom. Therefore, the intensive chlorine mass 35 of the two ^{15}N-isotope-labeled internal standards is disturbed by the chlorine mass 37 of the two analytes DPC and MDPC, at least for higher concentrations (> 4 µg/L). Therefore, it was necessary to use the 1/3 less sensitive chlorine mass 37 for the determination of isotopically labeled internal standard instead of the chlorine mass 35. The MS/MS detection conditions (cone voltage, CID energy and flow rate of nitrogen gas) were also optimised to obtain the highest signal intensities. Initial studies during the chromatographic method development had shown an unusually strong influence of matrix components, in particular, early eluting salts, on the measurement of the two chloridazon degradation products. Therefore, chromatography was optimised to enhance the separation of the target compounds from the early eluting matrix [9, 17]. Due to the polar nature of DPC and MDPC, the retention of both compounds on a regular C_{18}-LC column is not sufficient to separate the compounds from co-eluting matrix components [19]. For this reason, columns were examined with a low carbon loading or embedded polar groups. In addition, a nearly 100% aqueous initial gradient was used to increase the retention of the polar target analytes. The best performance was shown by the fully end-capped BEH C_{18}-column from Waters. The ethylene-bridge-hybrid technology improves the hydrolytic stability and allows to use a 100% aqueous mobile phase [20, 21]. A nearly 100% aqueous initial gradient has the advantage that the substance is focused on the head of the column (on-column focusing), which is useful for large volume injections [22, 23]. Therefore, it was possible to use an injection volume of 50 µL (this is important to achieve a LOQ in the low ng/L range) with a 2.1 x 50 mm column with 1.7 µm particle size (empty volume of the column was 170 µL). However, by injecting such a large volume the peaks were very broad. For this reason, the organic fraction of the mobile phase was

increased immediately after the injection. This resulted in very reproducible, sharp and intense peaks with peak widths below 0.15 sec and a retention factor k of 2.2 for DPC and 2.5 for MDPC. Under the described UPLC-MS/MS conditions, the complete chromatographic run took four minutes.

The influence of formic acid as an additive in the mobile phase was investigated. It is known that such additives increase the response of the measured substances because they are good proton donors during the positive electrospray ionisation process [11]. The maximum response for DPC and MDPC was obtained with formic acid at 0.01% v/v (2.7 mmol/L) in both mobile phases (shown in Figure 3-2). As discussed in detail below, the investigations have shown a strong matrix effect caused by frequently occurring salts in natural waters. The signal intensity of DPC and MDPC was particularly enhanced by ammonium, in contrast to the other investigated cations (Na^+ or K^+) (see Figure 3-5a). This effect was further studied in order to improve the sensitivity during the quantification of the two substances. To this end, a post-column infusion of ammonium ions via a mixing tee was used as a so-called mobile phase additive to the mobile phase. In this context, for example, TRIS as proton acceptor was described in the literature to increase the response during negative electrospray ionisation [24, 25]. In contrast, post-column addition of ammonium ions to enhance positive electrospray ionisation has not yet been described. To avoid negative effects of a non-volatile counter anion diluted ammonia solution was used as a volatile source of ammonium. The investigations showed that not only the concentration of ammonia (concentrations in total flow: 0.00, 0.14, 0.29, 0.58, 0.86, 1.15 mmol/L and concentrations of solutions: 0, 5, 10, 21, 31, 41 mmol/L) in the post-column infusion solution is crucial (Figure 3-3a), but also the flow rate of the infused ammonia solution to the mobile phase (Figure 3-3b). Based on these results, the investigation of different flow rates was conducted with water instead of ammonia solution. Here an increase in intensity was also observed, however less significant than with the ammonia solution (data not shown). This behaviour could be attributed to the fact that electrospray ionisation is also strongly influenced by the flow rate and therefore by the spray geometry during post-column infusion of modifier [26]. Finally, the greatest intensity was obtained at a concentration of 10 mmol/L in the ammonia solution at an injection flow of 25 µL/min into the mobile phase (0.7 mmol/L

ammonia in total in the mobile phase). The resulting signal enhancement by post-column addition of ammonia solution was approximately tenfold for both substances.

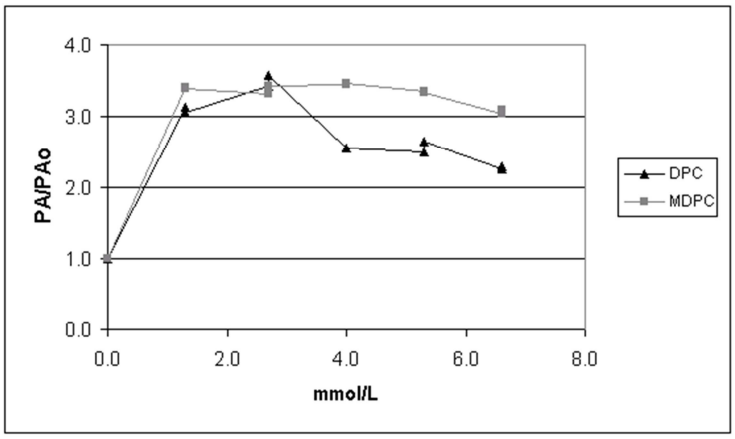

Figure 3-2: Results (mean of duplicate measurements) show a signal enhancement in the measurements of 1 µg/L DPC and MDPC during the addition of the respective amount of formic acid. The x-axis includes the added concentration of formic acid, while the y-axis shows the ratio of C/C_o. Here C_o represents the measurement concentration of DPC and MDPC without addition of formic acid and C the measurement concentration after addition of the respective concentration of formic acid.

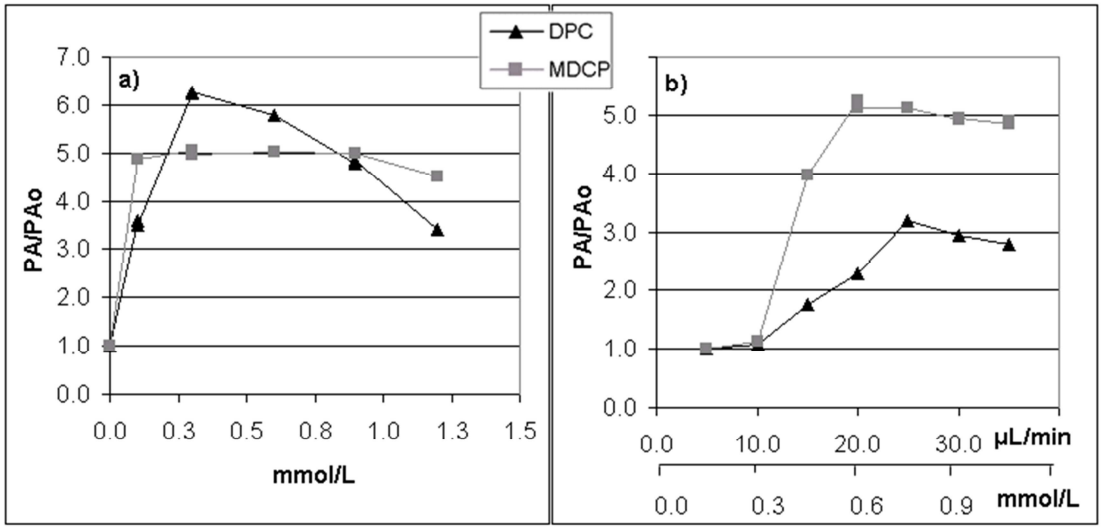

Figure 3-3: Results show a signal enhancement during the measurements of 1 µg/L DPC and MDPC (a) after post-column infusion of different concentrations of ammonia into the mobile phase with a constant flow of 15 µL/min. (b) After post-column infusion of constant concentrations (10 mmol/L solution) of ammonia with different flow rates (0 - 35 µL/min in 5 µL/min steps) into the mobile phase. X-axis includes added concentration of ammonia (a) or different flow rates respectively concentrations of ammonia in total flow (b). Y-axis shows also the ratio of C/C_o. Here C_o represents the concentration of DPC and MDPC without post-column infusion of ammonia solution and C the concentration after post-column infusion of ammonia solution.

3.4.2 Synthesis of the internal standard

Due to the strong influence of the matrix (see below), internal standards were necessary for the accurate quantification of DPC and MDPC. At the time of our study, only the isotopically labeled internal standard for DPC was commercially available (in mid-2011 a deuterium labeled internal standard for MDPC became commercially available), thus the synthesis of isotopically labeled MDPC was necessary. The only difference between DPC and MDPC is in the methyl group bound to the nitrogen in the ring system. This nitrogen belongs to an amide functional group, which has similar characteristics as a carboxylic acid in terms of reactivity towards methylating reagents. Therefore, the methylation with diazomethane was selected. This procedure is routinely used in laboratories in micro scale to prepare methyl esters of carboxylic acids for subsequent gas-chromatographic determination. The synthesis of internal standard was successfully with approximately 100% yield.

3.4.3 Validation

The validation procedure was carried out to determine the linearity, precision (intraday and interday), LOD, LOQ and the accuracy of the method. For the quantitative analysis of DPC and MDPC, the calibration range was from 0.01 to 1 µg/L. The linearity of response for DPC and MDPC was investigated by reference solutions in pure water. For both substances, very good correlation coefficients (R) were obtained (0.9994 for DPC and 0.9999 for MDPC, respectively). The intraday precision values, expressed as relative standard deviations (%RSDs) were lower than 5% for DPC and 3% for MDPC at two different concentration levels of 100 ng/L and 1 µg/L (repeated 10 times). For determination of interday precision, a 100 ng/L standard solution was used before and after the quantification of routine water samples in the same sequence. Interday precision obtained over 10 days, expressed as %RSD, was 11% for DPC and 4% for MDPC, respectively. The accuracy of the method was defined as the recovery in percent of the known added amount of substances in the sample. Therefore, DPC and MDPC were added to ten routine samples (added amount equivalent to a concentration of 250 ng/L). The recovery of the known added amount was in the range 99 - 110% for DPC and 99 - 108% for MDPC. The LOQ was defined as a signal to noise (S/N) ratio of 10:1 and the LOD as a S/N ratio of 3:1. The chosen S/N-ratios of LOQ and LOD values are typical for this kind of applications [4, 18, 21]. The LOD was determined in ultra pure water and also in real samples (Figure 3-4). For both DPC and MDPC the LOD and LOQ values were 0.01 µg/L and 0.03 µg/L, respectively.

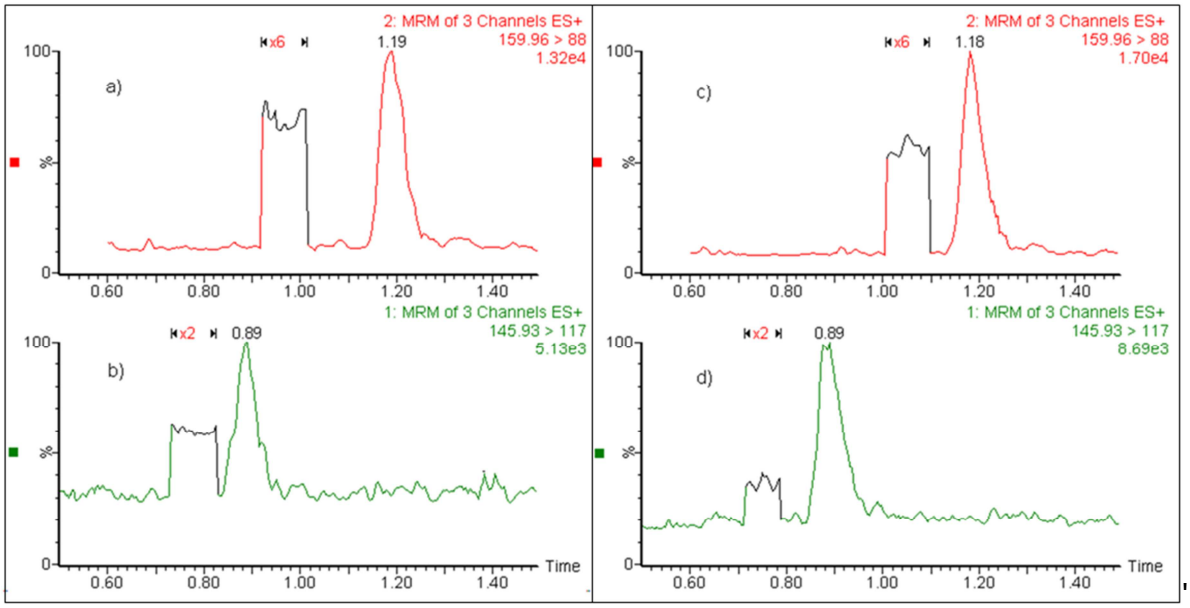

Figure 3-4. Response of DPC (b, d) and MDPC (a, c) at a concentration of 10 ng/L, both substances were determined in either ultra pure water (a, b) or a real water (c, d). For a better overview of the S/N-ratio, the noise level was zoomed (before DPC or MDPC peak) two times for DPC and six times for MDPC.

3.4.4 Matrix effects in artificial water samples

Investigations of the impact of matrix on the electrospray ionisation of DPC and MDPC were carried out in different specially designed artificial waters with various spiked inorganic salts and humic acid. Furthermore, possibilities to reduce matrix effects and the adequacy of the internal standards for quantification were studied.

Due to the polar character of both substances they are eluted very early from the column and the electrospray ionisation process for DPC and MDPC is affected by early eluted matrix components. The signal is thereby greatly enhanced or suppressed, thus preventing an accurate quantification [9, 17]. Early eluted compounds in real water samples are usually salts or humic substances, that occur in the aquatic environment and whose concentrations and composition vary greatly [9, 27]. To estimate the composition and concentration of salts and humic acid in the aquatic environment, data of 600 environmental samples (ground water, surface water and drinking water) were evaluated in the first instance with regard to the most frequent matrix substances and their highly concentrations. The 95%-quantile values of this concentrations were calculated to determine the high concentrations, however,

exclude extreme; these concentrations were used for further experiments. In addition, two further artificial solutions with only 50% and 10% of the 95%-quantile salt concentration have been investigated. The resulting salt concentrations used in this work are summarised in Table 3-3.

The following Figure 3-5 and Figure 3-6 show the influence of different matrix components on the response of the two measured analytes. The y-axis shows the deviations in percent of the target concentration (1µg/L), and the x-axis the relative concentrations of artificial matrix contents (100% = concentration in Table 3-3). The initial study was conducted without post-column infusion of ammonia modifier, therefore, it resulted without a signal enhancement or compensation of the matrix effects. The influence of salts (contained in artificial samples) on the response is very strong for both target compounds, the signals are significantly higher (> 500%) than measured in pure water (shown in Figure 3-5a and Figure 3-5b). An increase in the concentration of the salts (10%, 50% or 100%) usually leads to an increase in the response, with saturation occurring at higher concentrations. For DPC in the presence of sulphate, the response even decreases at high concentrations. In a comparison of the different salts, the matrix effect of ammonium is in most cases higher than that of sodium or potassium. This is particularly pronounced for sulphates that contain two positive charges.

The increase of intensity through the matrix was studied closer in terms of (i) a potential improvement of the sensitivity of the whole analytical method (see above) and (ii) an overall reduction of the matrix influence due to the controlled addition of an excess amount of a matrix component (in this case: ammonium). As a source of ammonium an ammonia solution was used, which was introduced post-column into the mobile phase. First, the post-column infusion of ammonia has been optimised to increase DPC and MDPC response (see optimisation chapter). The subsequent investigations of the influence of matrix were similar to those done previously, only that post-column addition of ammonia in the mobile phase was used.

The results are shown in Figure 3-5c and Figure 3-5d. For DPC and MDPC the whole matrix effect was much reduced. Especially for MDPC only 20% deviation from the target value was obtained for all matrix contents after the post-column infusion of

ammonia. Also the higher intensity caused by ammonium in comparison to sodium and potassium ions is no longer significant.

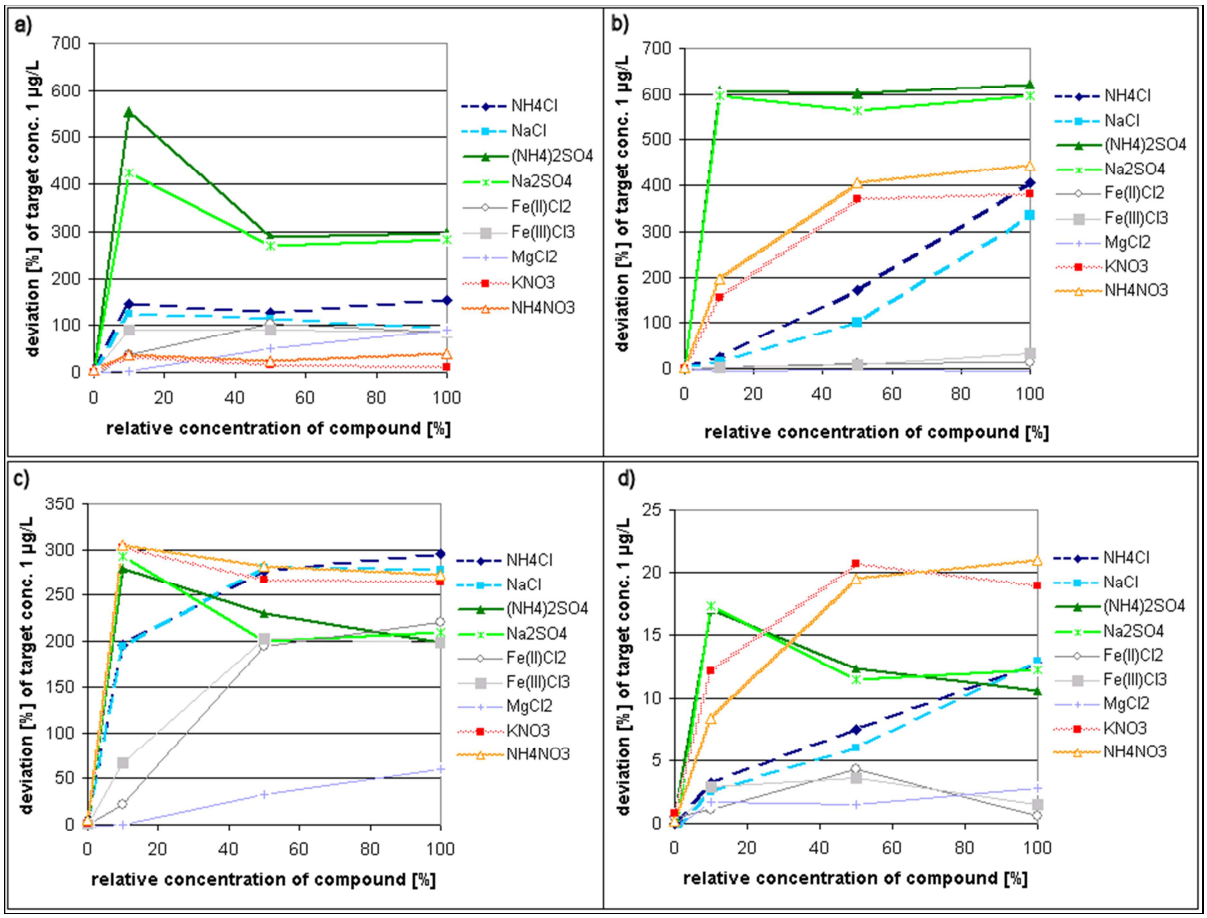

Figure 3-5: Influence of different matrix components on the response of (a, c) DPC and (b, d) MDPC, both at a concentration of 1 µg/L. Signal responses were measured without (a, b) and with (c, d) post-column addition of ammonia solution.

The post-column infusion of ammonia could not completely eliminate the matrix effect on the quantification of DPC and MDPC, therefore additionally an internal standard was used to compensate the remaining matrix effects. The effect of the additional use of isotopically labeled internal standards is shown in Figure 3-6 with (c/d) and without (a/b) post-column infusion of ammonia solution. In both cases, matrix effects for DPC and MDPC were very well compensated by application of the internal standard calibration, however, with post-column infusion of ammonia solution (Figure 3-6 c/d), the deviation from the target value is significantly lower. This is due to the reduction of matrix effects and the ten times higher sensitivity in case of post-column ammonia addition.

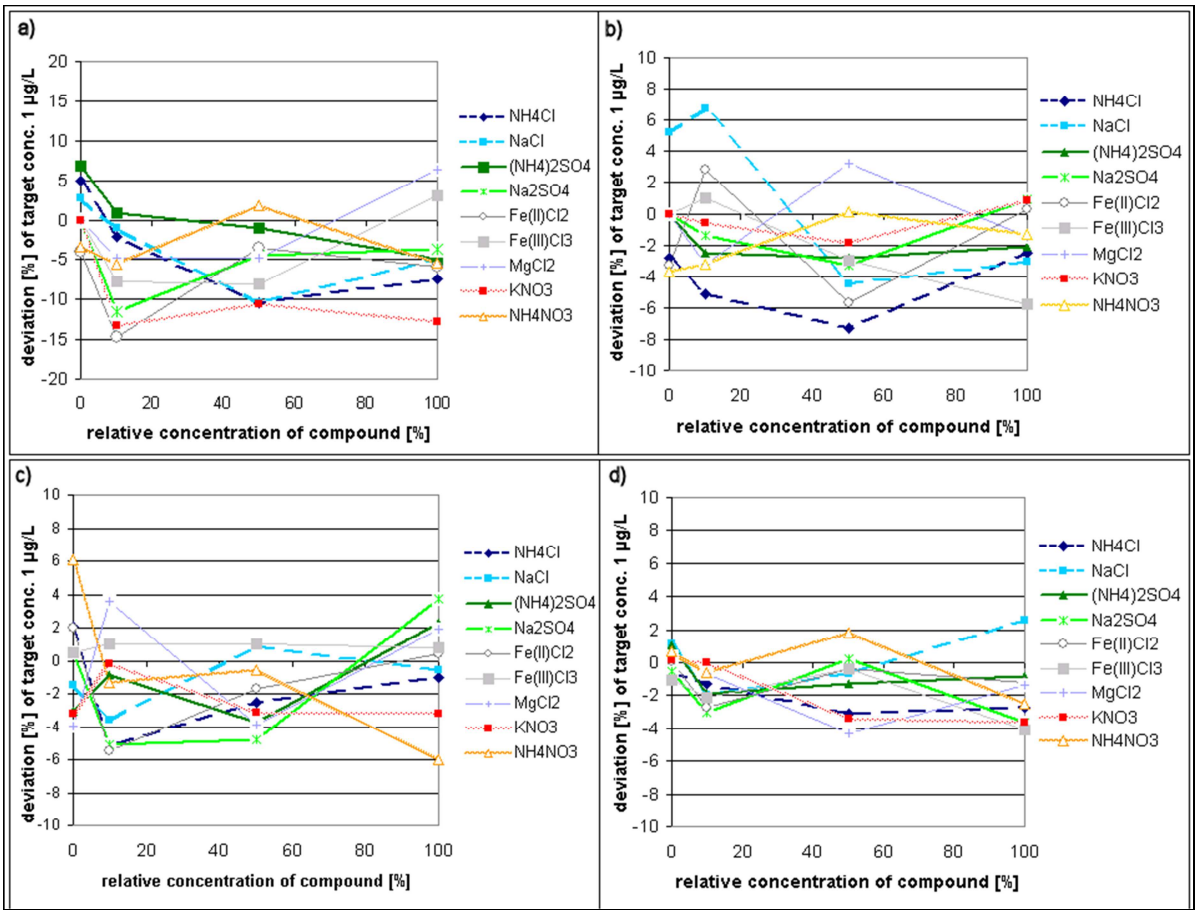

Figure 3-6: Compensation of matrix effects by internal standard (a, b) and additional use of post-column addition of ammonia solution (c, d) for DPC (left) and MDPC (right), both at a concentration of 1 µg/L.

3.4.5 Method robustness and suitability for routine analysis

In general, it is assumed, that standard addition provides the most accurate value of a substance concentration and is the most effective albeit laborious way to eliminate any matrix effect [12, 28-30]. For a comparison of calibration methods, various real samples (ten groundwater, five surface water and five drinking water samples) were prepared with the internal standard calibration as well as a standard addition method. This was done in order to cross-check quantitative results obtained with both approaches. Figure 3-7 shows that the concentrations quantified by the use of internal standard calibration are comparable with those obtained by standard addition method. DPC and MDPC concentrations obtained by the internal standard calibration

differ by less than 8% in comparison to concentrations measured by standard addition method.

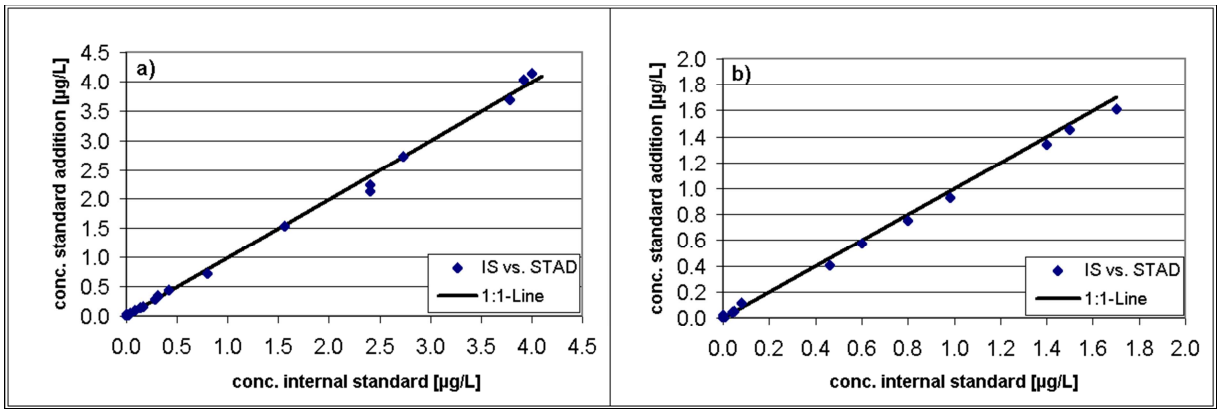

Figure 3-7: Comparison of the internal standard calibration (IS) vs. standard addition procedure (STAD) for quantification of DPC (a) and MDPC (b) in various real samples.

3.4.6 Routine measurements

With the post-column infusion of ammonia and internal standard calibration method, more than 1000 water samples have been measured since mid-2007, particularly in the Rhine and Ruhr region of North Rhine-Westphalia (Germany) and in the European Union. Figure 3-8 summarizes the statistical data derived from the monitoring of the different water bodies in box plots. Approximately 76% of all samples contained DPC and 62% contained MDPC. Over 70% of samples showed DPC concentrations ≥ 0.10 µg/L while 53% showed MDPC. Few groundwater samples contained higher concentrations up to 8 µg/L of DPC and 2.3 µg/L of MDPC respectively. The 90-percentile value was 3.1 µg/L for DPC and 0.77 µg/L for MDPC. Participation in a national round robin test, which was conducted in 2009 with 38 participating laboratories, led to successful results. For DPC a deviation ≤ 8% was obtained, while the MDPC deviation was ≤ 3% [15].

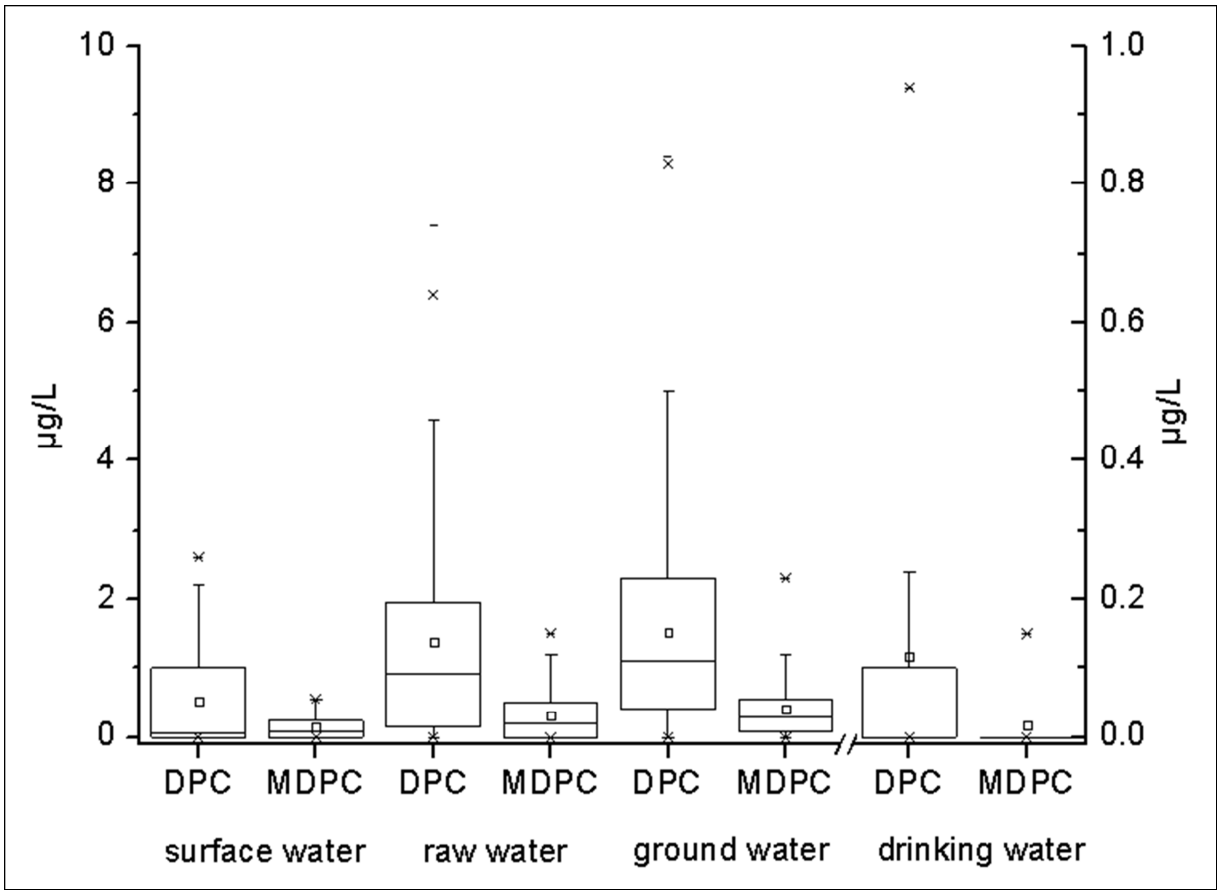

Figure 3-8: Box-whisker plots showing DPC and MDPC occurrence in various environmental water samples. The line in boxes represents the median, the boxes the 25 - 75% percentile, the whisker extends to the 95%-percentile of measured concentrations. The stars indicate the highest concentrations. The y-axis includes two different concentration ranges. The left y-axis refers to the concentration in surface water, raw water, and ground water, and the right y-axis to the concentration in drinking water.

3.5 Conclusions

The present study demonstrates a sensitive, accurate, and reliable analytical method for the quantification of the two polar degradation products desphenyl-chloridazon and methyl-desphenyl-chloridazon at trace levels in different water bodies. The robustness was examined in specially designed artificial and real water samples applying two different quantification methods (internal standard vs. standard addition method). The experiments showed a very strong influence (matrix effect) caused by different salts on the electrospray ionisation of DPC and MDPC. It was also shown that the post-column addition of an ammonia solution as additive may partly reduce matrix effects and additionally increases the sensitivity of the analytical method. Therefore post-column infusion of ammonia in combination with the use of isotopically labeled internal standards proved to be the best solution for the determination of DPC and MDPC in different water bodies. Furthermore, the increase of intensity by post-column infusion of additive, in this case ammonia solution, was also observed in own measurements for other substances that contain an amide or amino group. Here are just a few examples of investigated plant protection products, where an increased intensity was observed: bromacil, chloridazon, chlorotoluron, diuron, isoproturon, metazachlor and metolachlor. Thus, it is proposed that this effect may improve in general the ESI-LC-MS (positive ESI-mode) detection of compounds incorporating such functional groups. Finally, a national round robin test was passed very well with a deviation between 3 - 8%.

3.6 References

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Chapter 4. Fully automated standard addition method for the quantification of 29 polar pesticide metabolites in different water bodies using LC-MS/MS*

* *Submitted to Journal Analytical Bioanalytical Chemistry, in revision.*

4.1 Abstract

A reliable quantification by LC-ESI-MS/MS as the most suitable analytical method for polar substances in the aquatic environment is usually hampered by matrix effects from co-eluting compounds, which are unavoidably present in environmental samples. The standard addition method (SAM) is the most appropriate method to compensate matrix effects. However, when performed manually this method is too labour- and time-intensive for routine analysis. In the present work, a fully automated SAM using a multi-purpose sample manager “Open Architecture UPLC®-MS/MS” (ultra performance liquid chromatography tandem mass spectrometry) was developed for the sensitive and reliable determination of 29 polar pesticide metabolites in environmental samples. A four-point SAM was conducted parallel to direct-injection UPLC-ESI-MS/MS determination that was followed by a work flow to calculate the analyte concentrations including monitoring of required quality criteria. Several parameters regarding the SAM, chromatography and mass spectrometry conditions were optimised in order to obtain a fast as well as reliable analytical method. The matrix effects were examined by comparison of the SAM with an external calibration method. The accuracy of the SAM was investigated by recovery tests in samples of different catchment areas. The method detection limit was estimated to be between 1 – 10 ng/L for all metabolites by direct injection of a 10 µL sample. The relative standard deviation (%RSD) values were between 2 - 10% at the end of calibration range (30 ng/L). About 200 samples from different water bodies were examined with this method in the Rhine and Ruhr region of North Rhine-Westphalia (Germany). Approximately 94% of the analysed samples contained measurable amounts of metabolites. For most metabolites low concentrations ≤ 0.10 µg/L were determined. Only for three metabolites, the concentrations in ground water were significantly higher (up to 20 µg/L). In none of the examined drinking water samples the health related indication values (HRIV are between 1 - 3 µg/L) for non-relevant metabolites were exceeded.

4.2 Introduction

Currently, about 250 pesticide active ingredients in more than 900 different commercial plant protection products (PPP) are approved in Germany (status 2010) [1]. The annual sales volume of PPP has increased since 1994 until in recent years a constant annual sales volume of about 35,000 tons of PPP has been reached in Germany [2]. Moreover, the spectrum of active ingredients has changed in the last 50 years to more polar and more degradable substances [3]. PPP are primarily utilised in agriculture (80%); other fields of application are the treatment of railway tracks, municipal areas, and private use (20% in total). The application is done several times per year and over large areas. Especially when applied incorrectly, pesticide active ingredients – or frequently their metabolised products – may directly enter surface water and ground water by leaching and drainage processes [4]. The metabolites are usually more polar and persistent than the original pesticide active ingredients, therefore such substances are not effectively removed during water treatment processes, e.g. by bank filtration or activated carbon adsorption. Furthermore, if oxidative water purification processes are used, substances of toxicological concern may be formed. This has been demonstrated for the case of N,N-dimethylsulfamide (DMS), a metabolite of the fungicide tolylfluanid. DMS has been detected for the first time in significant concentrations in ground water in different regions of Germany at the end of 2006. The use of certain operational settings of an ozonation process during the water treatment process can lead to the conversion of even small amounts of DMS to the carcinogenic N-nitrosodimethylamine (NDMA) [5]. The outdoor application of tolylfluanid was therefore banned in early 2007. Due to reports of further hitherto unknown metabolites and the pressure of the public, the German Federal Office of Consumer Protection as well as Food Safety (BVL) and Federal Environment Agency (UBA) published in 2008 data about so-called non-relevant metabolites – that have been found in rather high concentrations of 1 - 10 µg/L – during lysimeter tests (Table 4-1) [1, 6, 7]. Metabolites are called non-relevant when they have lost the origin pesticide activity and are devoid of toxic or genotoxic potential [8]. Additionally, the UBA has proposed two health related indication values (HRIV) as the basis for the assessment of non-relevant metabolites in ground water [9]. Previous sampling campaigns of individual metabolites show their frequent

occurrence in ground water and drinking water everywhere in Germany [10-14]. Consequently, it is important to monitor these substances in the aquatic environment with a reliable and sensitive analytical method, to respond immediately if changes occur in water quality. The most suitable analytical method for the determination of polar pesticides or their metabolites in environmental analysis is direct-injection LC-ESI-MS/MS due to the simple sample preparation and high sensitivity [11]. However, many environmental water constituents such as inorganic salts or humic substances, especially if they co-elute with the target analytes, affect the ionisation efficiency during measurement [12, 15]. The presence of such co-eluting matrix components leads frequently to ion suppression/enhancement effects, so-called matrix effects [16-20]. These matrix effects occur very often during quantitative analysis using LC-ESI-MS/MS and affect accuracy, precision and limit of detection [21]. Therefore, the analysis in complex matrices needs further reduction or compensation of matrix effects. This is particular the case in biological samples and food or if sample matrix varies because of different composition of environmental samples or water bodies [12, 22, 23]. To ensure an accurate quantification of the target analytes, different routinely used calibration methods for multi-component analysis were proposed in order to compensate the matrix effects.

The external calibration with matrix-matched standards is often suggested in literature [24], however, this method offers only an insufficient compensation with diluted samples in a uniform matrix [23]. Another disadvantage of the method is that in the case of variable sampling areas or water bodies, the availability of appropriate matrix-matched blanks free of residues of the target analyte is not guaranteed. Therefore, the matrix-matched method is not suitable for the quantification of metabolites in different water bodies. The isotopically labeled internal standards (IS), with an equal behaviour as the target analytes in different sample matrix - during chromatography and ionisation process - are used frequently to compensate the matrix effects [25-27]. However, isotopically labeled IS are not commercially available for new metabolites. Only the standard addition method (SAM) is considered as the accurate alternative to compensate diverse matrix effects [18, 23]. SAM ensures an in-situ real matrix matched calibration and allows as the only method an internal control for the quantification. This method also allows the simple investigation of the

matrix effects by comparing the slopes of calibration curves (SAM vs. external calibration method) [28]. However, it is frequently reported that the SAM is labour-intensive and time-consuming because multiple addition and analysis of each sample is needed. Therefore, it was necessary to develop an automated SAM procedure and fast multi-component LC-MS determination for the quantification of 29 metabolites in various water samples (Table 4-1). Accordingly, a multi-purpose autosampler equipped with a sample handling function is used for the automation of a manual four-point SAM. The direct sample injection in connection with ultra fast chromatography (UPLC) and triple quadrupole mass spectrometer allows fast separation and detection needed for the multi-component determination.

Aims of this work were (i) the optimisation of automated sample handling steps parallel to the chromatographic separation and mass spectrometric detection in order to obtain a fast and reliable analytical method without carry-over effects. (ii) Development of a work flow of the whole SAM, starting with the measurement via data evaluation and calculation of target concentrations. The work flow contains additionally the application of quality criteria (linearity, precision and confidence interval) on each determination. (iii) Validation and verification of the robustness in different environmental samples. (iv) Application of the SAM for determination in real water samples.

Table 4-1: Overview of the investigated pesticide metabolites, their log P values (simulated [29]), health related indication values (HRIV), and maximum concentrations in lysimeter tests [1, 7, 30, 31].

Nr.	Metabolite	Molar mass (g/mol)	Application rate during lysimeter tests (kg/h)	Maximum concentrations in lysimeter water (µg/L)	Partition coefficient (log P)	Health orientation value (µg/L)
1	N,N-dimethylsulfamide	124.2	--	--	-1.54	1
2	desphenyl-chloridazon	145.5	2.50	40.6	-0.78	3
3	methyl-desphenyl-chloridazon	159.6	2.50	2.1	-0.55	3
4	2,6-dichlorbenzamide	190.0	--	94.0	2.03	3
5	flufenacet-OA	225.2	--	--	1.95	--
6	flufenacet-M2	275.3	0.48	1.6	1.19	1
7	dimethenamid-P-M23	271.3	1.39	1.0	2.34	1
8	dimethenamid-P-M27	343.4	1.39	4.0	1.58	1
9	dimetachlor-CGA369873	265.3	1.50	2.3	1.44	1
10	dimetachlor-CGA354742	323.3	1.50	35.1	1.25	3
11	dimetachlor-CGA50266	251.3	1.50	36.2	2.00	3
12	chlorthalonil-M5	268.5	2.50	1.4	2.29	3
13	chlorthalonil-M12	329.6	0.99	10.3	-0.68	3
14	metazachlor-BH479-4	273.3	1.00	21.4	2.05	1
15	metazachlor-BH479-8	323.4	0.96	17.0	0.35	3
16	metazachlor-BH479-9	349.4	0.96	1.3	1.01	3
17	metazachlor-BH479-11	305.4	0.96	2.5	1.19	3
18	metazachlor-BH479-12	303.3	0.96	3.6	1.09	3
19	S-metolachlor-CGA351916	279.4	1.25	16.3	2.88	3
20	S-metolachlor-CGA-380168	351.4	1.25	28.0	2.11	3
21	S-metolachlor-CGA368208	279.3	1.25	7.8	1.88	1
22	S-metolachlor-CGA357704	279.3	1.25	5.1	--	1
23	S-metolachlor-NOA413173	373.3	--	3.0	--	1
24	metalaxyl-M-CGA62826	265.3	0.16	6.9	1.97	1
25	metalaxyl-M-CGA108906	295.3	0.16	1.9	1.12	1
26	quinmerac-BH518-2	251.6	0.24	2.4	1.51	1
27	tritosulfuron-BH635-4	353.3	0.05	1.0	0.25	1
28	dimoxystrobin-505-M08	356.4	0.25	2.4	0.70	3
29	dimoxystrobin-505-M09	356.4	0.25	2.0	0.70	3

4.3 Experimental

4.3.1 Reagents and materials

The pesticide metabolites were chosen based on information available from the UBA published 2008/2009. Not for all metabolites reference standards were commercially available. In these cases, they were obtained directly from the crop agent manufacturer. N,N-dimethylsulfamide (DMS), purity 98%, was achieved from ABCR GmbH & Co.KG (Germany). Desphenyl-chloridazon (DPC) and methyl-desphenyl-chloridazon (MDPC) dissolved in acetonitrile (100 mg/L) with a purity of 99% were purchased from Dr. Ehrenstorfer GmbH (Germany). Flufenacet -OA, -M2 (ESA), S-metolachlor- CGA368208, -CGA357704 and -NOA413173 all with a purity greater than 99% were obtained from Sigma-Aldrich Co. (USA). The 2,6-dichlorbenzamide, purity 99.8% was from Riedel-de Haen AG (Germany). The following standards of pesticide metabolites were obtained directly from the crop agent manufacturer. The purity is denoted in brackets. Dimethenamid-P-M23 [98.8%], -M27 [91.1%]; dimoxystrobin-505M08 [97.8%], -505M09 [93.3%]; metazachlor-BH479-4 (OA) [98.6%], -BH479-8 (ESA) [99.1%], -BH479-9 [94.8%], -BH479-11 [99%], -BH479-12 [97.3%]; quinmerac-BH-518-2 [99.2%] and tritosulfuron-BH-635-4 (635M01) [94.5%] were from BASF SE (Germany). Chlorothalonil-M5 (611965) [96%], -M12 (417888) [89%]; dimethachlor-CGA50266 [90%], -CGA354742 [93%], -CGA369873 [99%]; metalaxyl-M-CGA62826 (NOA409045) [99%], -CGA108906 [99%]; S-metolachlor-CGA380168/CGA354743 racemate [96%] and -CGA351916/CGA51202 racemate [97%] were from Syngenta Crop Protection AG (Germany). The LC-MS grade acetonitrile, water, and formic acid were obtained from Biosolve (Netherlands). TRIS Tris(hydroxymethyl)aminomethan ultrapure was from VWR International GmbH (Germany) and ammonia solution (25%, analysis grade) was from Merck KGaA (Germany). The water purification system Seralpur Pro 90 CN (Germany) was used for ultrapure water required for the dilutions. Argon gas (5.0) for the collision cell was obtained from Air Liquide (Germany). A CMC instruments gas generator (Eschborn, Germany) was employed for preparing nitrogen gas for the mass spectrometric system. Disposable nylon syringe filters, pore size diameter 0.2 µm, were from Pall Life Sciences (USA). 2-mL polypropylene syringes for the filters were obtained from

Terumo (Slovakia). Glass syringes (100, 250, 500 µL) were from Hamilton Bonaduz AG (Switzerland).

4.3.2 UPLC-ESI-MS/MS analysis

Analyses were performed on a Waters Acquity UPLC®-Xevo TQ-S ultra performance liquid chromatography coupled with an electrospray ionisation tandem mass spectrometric system (Milford, USA). The Waters Open Architecture UPLC® 2777C system was used as a programmable multi-purpose sample manager for sample injection and liquid handling needed for the SAM. The mass spectrometric operating parameters were optimised using an infusion system with a T-junction. Each standard (100 µg/L) was infused with a flow rate of 10 µL/min using one line of the T-junction, and 0.1% formic acid in acetonitrile and water (5:95, v/v, mobile phase) was added at 500 µL/min using the second line. The desolvation temperature was optimised from 350 to 650°C, in 50°C steps. The final ionisation source parameters used for positive and negative ESI mode were: nitrogen as drying gas (1000 L/h); capillary voltage +/- 0.8 kV, collision gas argon (0.15 mL/min) and 650°C desolvation temperature. The cone and collision-induced dissociation (CID) were adapted for each substance. The protonated/deprotonated adducts were used as precursor ions and two MS/MS transitions were acquired for each analyte, using the intensity ratio as confirmatory parameter. The m/z values of the precursor ions, product ions, and the CID energy for the quantification transitions in the selected reaction monitoring (SRM) mode are listed in Table 4-2.

Table 4-2: Settings of the tandem quadrupole for the precursor–product transitions, retention times, and k-factors.

Analyte	Precursor ion (m/z)	1st product ion, quantifier (m/z)	2nd product ion, qualifier (m/z)	Cone (V)	1st and 2nd collision energy (eV)	Time (min)	k-factors
N,N-dimethylsulfamide	125.0	108.0	79.9	20	20 / 10	1.38	2.8
desphenyl-chloridazon	146.0	117.0	54.1	2	18 / 20	1.47	2.9
methyl-desphenyl-chloridazon	159.9	87.9	130.2	2	24 / 22	1.85	3.7
2,6-dichlorbenzamide	190.0	108.9	74.1	2	38 / 32	2.79	5.6
flufenacet-OA	226.1	138.0	180.1	2	16 / 8	2.94	5.9
flufenacet-M2	276.1	112.1	216.1	2	22 / 18	3.17	6.3
dimethenamid-P-M23	272.1	240.1	126.0	18	30 / 12	3.46	6.9
dimethenamid-P-M27	322.1	290.1	210.1	30	22 / 14	3.41	6.8
dimetachlor-CGA369873	244.0	122.1	144.1	16	14 / 18	2.47	4.9
dimetachlor-CGA354742	302.1	270.1	174.2	30	28 / 14	3.1	6.2
dimetachlor-CGA50266	252.1	220.1	105.2	2	30 / 12	2.89	5.8
chlorthalonil-M5	268.0	178.9	167.0	30	30 / 32	2.23	4.5
chlorthalonil-M12	328.7	221.9	219.9	40	24 / 25	2.82	5.6
metazachlor-BH479-4	274.1	162.1	69.0	2	10 / 8	2.91	5.8
metazachlor-BH479-8	324.1	134.1	69.0	4	10 / 26	3.01	6.0
metazachlor-BH479-9	350.1	134.1	105.1	4	50 / 18	3.13	6.3
metazachlor-BH479-11	306.1	134.1	238.1	6	16 / 8	3.48	7.0
metazachlor-BH479-12	304.1	69.0	160.1	4	8 / 26	2.51	5.0
S-metolachlor-CGA351916	280.2	248.2	146.1	2	24 / 12	4.25	8.5
S-metolachlor-CGA-380168	330.2	298.2	202.1	10	28 / 14	3.6	7.2
S-metolachlor-CGA368208	258.1	136.0	119.8	42	28 / 20	2.88	5.8
S-metolachlor-CGA357704	280.1	234.0	133.9	26	24 / 10	3.35	6.7
S-metolachlor-NOA413173	330.2	162.1	284.1	10	22 / 10	3.4	6.8
metalaxyl-M-CGA62826	266.1	220.0	192.2	20	16 / 14	4.12	8.2
metalaxyl-M-CGA108906	296.1	160.1	178.1	14	24 / 16	3.25	6.5
quinmerac-BH518-2	252.0	162.0	233.8	2	30 / 14	2.43	4.9
tritosulfuron-BH635-4	354.1	86.0	129.0	6	22 / 18	2.89	5.8
dimoxystrobin-505-M08	357.2	116.0	182.1	4	30 / 26	4.72	9.4
dimoxystrobin-505-M09	357.2	182.1	116.0	4	30 / 26	4.86	9.7

The following columns were investigated for separations: Acquity UPLC BEH C_{18} (2.1 x 100 mm), Acquity UPLC BEH Shield RP18, both with a particle size of 1.7 µm and Acquity UPLC HSS T3 (2.1 x 100 mm) with a particle size of 1.8 µm (Waters, Milford, USA). The optimisation of the chromatographic conditions comprised several temperatures (15 - 50°C) and gradient settings. Measurements at each parameter combination were repeated 10 times. Afterwards, conditions were chosen which

provided optimised retention for the compounds as well as lowest standard deviation and highest signal intensity. Finally, an Acquity UPLC HSS T3 (2.1 x 100 mm, 1.8 µm) column was used with an oven temperature of 40°C and water (A) / acetonitrile both with 0.01% formic acid (99.9:0.1% v/v) as the initial mobile phase (the used gradient is shown in Table 4-3). The flow rate was 500 µL/min and the total run time was 6.3 min. The programmable multi-purpose sample manager was used for the sample injection (10-µL loop), addition of the spiked standard solution, and sample mixing. The syringe was cleaned twice after each step with acetonitrile and water. For the SAM exactly 1 mL of sample was filled up manually in a 1.5 mL vial. The complete sample injection and SAM were carried out automatically by autosampler as follows: a sample volume of 10 µL was drawn from the vial and dispensed into the injection port followed by cleaning of syringe and injection port. Parallel to the LC-MS determination an aliquot volume (10 µL) of the standard solution was spiked from a reservoir to the same sample vial, which is by this addition filled up to 1 mL again. Followed by the sample mixing step, the sample was sucked and dispensed by the syringe 5 times. The nominal final concentrations in the sample were 100, 200, 300 and 400 ng/L. The complete SAM procedure contained 5 measurements (unspiked sample + four spiked concentrations) and took 32 min per sample. The specific standard addition steps are shown in Table 4-3.

Table 4-3: Chromatographic conditions (gradient table) and SAM steps during one run.

Time (min)	Flow rate (mL/min)	%A	Time (min)	SAM steps
0.0	0.5	99.9	0.0 - 1.0	Sample injection
3.5	0.5	60.0	1.0 - 1.4	Syringe cleaning (acetonitrile/water)
5.0	0.5	40.0	1.4 - 1.7	Add spike solution (10 µL)
5.2	0.5	1.0	1.7 - 2.1	Sample mixing (n = 5)
5.8	0.5	1.0	2.1 - 2.5	Syringe cleaning (acetonitrile/water)
6.1	0.5	99.9	2.5	Home position
6.3	0.5	99.9		

For the determination of metabolites in real samples, a valve switching mechanism was introduced that controlled the flow of the mobile phase to the detector to minimise the contamination of the instrument with non-volatile sample matrix. Only within the time frame when the metabolites were eluted, the mobile phase was

directed to the detector. Mobile-phase modifiers like NH_4OH (ESI positive mode) and TRIS (ESI negative mode) were examined by post column infusion. During the continuous monitoring of all substances, the modifier solution (20 mmol/L) was introduced with a constant flow of 20 µL/min through the T-junction into the mobile phase with a flow of 500 µL/min.

4.3.3 Sample preparation, reference solutions, validation and quantification

Stock standard solutions were prepared by weighing and dissolving the pure analytical standard in acetonitrile (stored at 4°C). These solutions were used to prepare diluted reference or spike solutions. Aqueous reference solutions for the external calibration were prepared by diluting the stock solutions with pure water to yield a concentration range of 0.05 - 0.8 µg/L, containing twelve calibration points. These solutions were used to check the linearity. Six levels (0.05 – 0.13 µg/L) were used to estimate the limit of quantification (LOQ), limit of detection (LOD), and three solutions (0.03, 0.25 and 0.8 µg/L) were measured in ten replicates in order to determine the relative standard deviation (%RSD). The spiked standard addition solution was also prepared by diluting the stock solution with ultrapure water to a final concentration of 10 µg/L. The solution was applied for the development and optimisation of the SAM and for the quantification of pesticide metabolites in real water samples. This solution was also added (100 ng/L) to 30 real samples (each 10 surface water, ground water and drinking water samples) to determine the recovery and examine the robustness of the method.

4.3.4 Sample preparation for routine measurements and SAM work flow

The real samples were collected from different areas in North Rhine-Westphalia and contained drinking water, ground water, deep well water, surface water, and waste water. The samples were stored at 4 - 6°C and measured within 2 weeks. First, particle-containing samples were filtrated by a syringe filter and transferred, after reaching room temperature, into a vial using an Eppendorf pipette (exactly 1 mL). For the determination of water samples, the following work flow was applied: First, two pure water samples were analysed in order to condition the column. Afterwards, reference solutions were measured, followed by using the SAM on 1 mL pure water

to check the spiked concentrations, which was used as an internal control of the stock concentrations. Additionally, by the comparison of calibration curve slope (blank vs. real sample) the matrix effect may be determined if required. Thereafter, the determination of real water samples by the SAM was carried out successively, as described above. The evaluation was carried out as an external calibration of the batch with 5 measurements (unspiked sample + four spiked concentrations). Peak areas were smoothed and integrated automatically by TargetLynx software, which is implemented in Waters master software (MassLynx 4.1, Milford, USA). The software displays the peaks, calibration curve and determines the coefficient of correlation, relative standard deviation, slope and abscissa. These values were exported as TXT-file (TargetLynx; LIMS export) for further calculations of the analyte concentration using Excel (Microsoft Office 2003, Microsoft Corporation, Redmond, USA). The calculations in Excel included the concentration and the confidence interval (f = 3; P = 95%) as described in German norm DIN 32633 [32].

4.4 Results and discussion

4.4.1 Optimisation of UPLC-ESI-MS/MS conditions

To identify the mass spectrometric operating parameters for each compound, the cone voltage as well as the collision induced dissociation (CID) energy were optimised by Auto-Tune-Wizard. For the majority of the compounds, the protonated molecule $[M+H]^+$ and only for the chlorthalonil-M12 the deprotonated molecule $[M-H]^-$ were chosen, because of higher intensity. The most sensitive mass transition was selected for quantification purposes and the other one was used for confirmation. More selective product ions were chosen instead of ions with unspecific losses (e.g. H_2O or CO_2) during the collision cell fragmentation process. The two metabolites of dimoxystrobin with isobaric interferences could be separated chromatographically. For all transitions a minimum dwell time of 20 ms and 15 points per peak were used to assure a reliable identification without any resolution or sensitivity loss. The desolvation temperature showed a strong influence on the ionisation process, therefore, the temperature range from 350 to 650°C was examined. Each increase of 100°C led to an intensity enhancement with an average of 40% for all metabolites. The highest intensity was achieved at maximum temperature of 650°C, with an intensity about 210% higher for all compounds compared with 350°C (see Figure 4-1), and was chosen for all experiments.

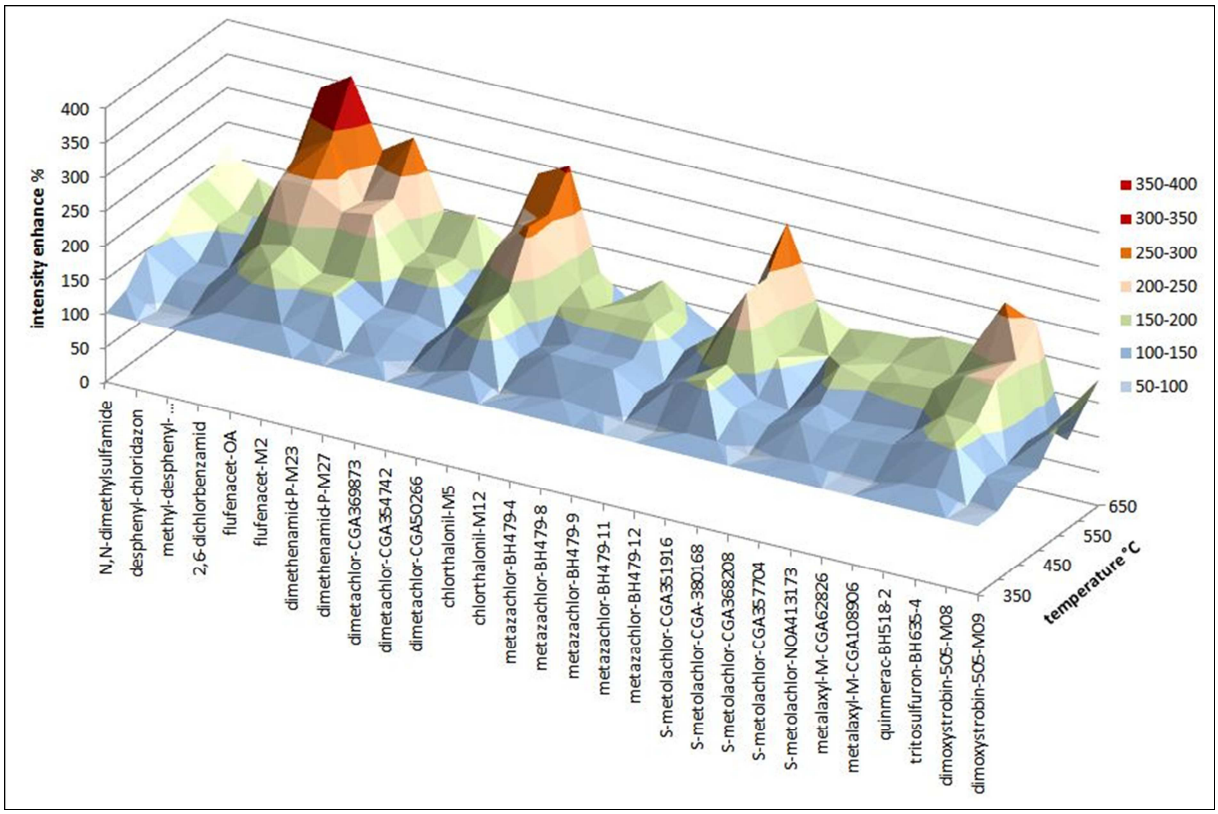

Figure 4-1: Influence of examined ionisation temperatures (350 - 650°C) on the response signal of metabolites. Y-axis indicates the intensity enhance in percent (ascending from 100%), x-axis shows the metabolites and z-axis the different temperatures.

Especially by using the triple quadrupole mass spectrometer, an adequate separation of all components is necessary in order to maintain a satisfying dwell time per peak and to separate substances that cause isobaric interferences, such as the two metabolites of dimoxystrobin. Additionally, many water constituents such as inorganic salts or humic substances affect the ionisation efficiency during the LC-ESI-MS/MS detection [15]. The presence of such matrix components leads to an increase or decrease of response intensity, the so-called matrix effect [16-20, 33]. Especially for polar compounds, because of insufficient retention in reversed-phase chromatography, this effect was very significant [15]. Previous studies have shown that for two of the chosen polar metabolites an unusually strong influence of the matrix of more than 600% signal increase was caused by salts as important constituents in environmental samples [12]. Therefore, the chromatography was optimised to enhance the separation of the target compounds from early eluting matrix constituents (e.g. salts) [17-20, 33]. Due to the polar nature of the metabolites, two columns suitable for polar substances were examined. A column with a low

carbon loading (HSS T3 C_{18}) and one with embedded polar groups (BEH Shield RP 18) were used. These columns were compared to an often used column (BEH C_{18}) concerning their reproducibility and long life time (used in our laboratory for more than 6000 direct injections of routine samples). In order to use the SAM a high reproducibility and durability of a column is important because multiple measurements per sample are needed. These columns were also chosen because the ethylene-bridge hybrid technology was reported to improve the hydrolytic stability and allows to use a 100% aqueous mobile phase [34, 35]. A nearly 100% aqueous initial gradient increases the retention of the polar analytes, and the substances are focused on the head of the column (on-column focusing) [36, 37]. The optimisation of the chromatographic conditions comprised, besides the three different column types, different gradients for the mobile phase and column temperatures. Each parameter combination was tested 10 times. The conditions with sufficient retention (k-factors > 2) and baseline separation between peaks, lowest standard deviation and highest signal intensity were selected for further measurements. The HSS T3 C_{18} column showed the highest retention in particular for early eluted analytes (k-factors 2.8 – 4) and between 12 - 15%, more retention was measured in comparison to the BEH C_{18} column. The BEH Shield RP 18 column has a significantly lower retention of 10 - 25% than the BEH C_{18} column regarding the early eluted analytes. In addition, DMS as the most polar metabolite shows a good peak symmetry only on the HSS T3 column and no peak broadening was observed as well. For analytes with higher k-factor 4 – 8, the BEH Shield RP 18 column exhibited a much higher retention in comparison to the BEH C_{18} column (30 - 140% higher k-factors). The HSS T3 column revealed a relative increase in retention of only 30% compared to the BEH C_{18}. Smallest standard deviations of peak areas were found for the BEH C_{18} column, while for the HSS T3 column the highest S/N ratios were determined, followed by the BEH C_{18} and BEH Shield RP 18 column with 30% and 48% lower S/N ratios at the same analyte concentration, respectively. Due to these findings, the UPLC HSS T3 (C_{18} 2.1 x 100 mm, 1.8 µm) column was finally chosen. The different column temperatures (15 - 50°C) had no effect on the chromatography. The selected gradient settings are given in Table 4-3 and the total run time was 6.3 min. The selected chromatographic settings are a compromise between fast separation that was necessary for reducing of the total analysis time per sample and overlapping of

peaks or decrease of intensity. Shorter analysis times by steeper gradients or shorter columns (50 mm) have led to more overlaps between target peaks and thereby short dwell times (< 10 ms) for mass spectrometric detection, resulting in significant loss of intensity. Therefore, only 100 mm columns were extensively investigated.

4.4.2 Influence of different mobile phase additives on signal response

It is known that different modifiers added to the mobile phase enhance the response during electrospray ionisation. For example, ammonium ion as proton donator or TRIS as proton acceptor was described in the literature to increase the response during positive or negative electrospray ionisation [12, 38, 39], respectively. In this context, a post-column infusion of ammonia in ESI positive mode and TRIS in ESI negative mode (each 20 mmol/L) into the mobile phase was applied to enhance the ionisation process. For 10 investigated analytes, the intensity of the response increased (10 - 250%) by the infusion of an ammonia solution. However, at the same time, the intensity of the remaining analyte decreased by about 30 - 100% (see Table 4-4). Infusion of TRIS led to a significant suppression (100%) of the signal and cannot be used. Therefore, for this multi-component method with 29 closely eluting analytes, with successive signal enhancement and suppression, the infusion of ammonia solution previously adopted in the analysis of two chloridazon degradation products cannot be applied [12]. However, ammonia solution could be used for certain substances that showed a signal enhancement when a lower LOD is needed.

Table 4-4: Influence of both examined modifiers on the peak areas of metabolites.

Analyte	Intensity (%)	
	Ammonia solution	TRIS
N,N-dimethylsulfamide	-63	-100
desphenyl-chloridazon	108	-100
methyl-desphenyl-chloridazon	157	-97
2,6-dichlorbenzamide	-63	-99
flufenacet-OA	-96	-100
flufenacet-M2	-36	-94
dimethenamid-P-M23	-80	-100
dimethenamid-P-M27	17	-94
dimetachlor-CGA369873	-41	-99
dimetachlor-CGA354742	63	-94
dimetachlor-CGA50266	-76	-100
chlorthalonil-M5	-100	-100
chlorthalonil-M12	-72	-99
metazachlor-BH479-4	-76	-100
metazachlor-BH479-8	25	-94
metazachlor-BH479-9	19	-84
metazachlor-BH479-11	182	1
metazachlor-BH479-12	-78	-99
S-metolachlor-CGA351916	-82	-100
S-metolachlor-CGA-380168	11	-94
S-metolachlor-CGA368208	-53	-93
S-metolachlor-CGA357704	-94	-100
S-metolachlor-NOA413173	-21	-92
metalaxyl-M-CGA62826	-31	-96
metalaxyl-M-CGA108906	-68	-98
quinmerac-BH518-2	-37	-100
tritosulfuron-BH635-4	-92	-97
dimoxystrobin-505-M08	256	-79
dimoxystrobin-505-M09	238	-37

4.4.3 Standard addition method work flow

In previous studies it was shown that the determination of polar pesticide metabolites by the use of UPLC-ESI-MS/MS is hampered by varying matrix effects, depending on the natural occurring substances in different water bodies [11, 12, 40]. The comparison of obtained concentrations by external calibration and SAM confirmed this result. The slopes of the calibration plots obtained by external calibration and SAM were different, which indicates a matrix effect [28]. As described in detail in the introduction other matrix effect compensation methods like IS and matrix-matched calibration are not possible. Therefore, SAM seems to be the most suitable method and was applied in this study. The frequently described disadvantages of SAM (labour-intensive sample-handling and time-consuming multiple measurements [24, 41, 42]) were solved by the use of a multi-purpose autosampler that handles the standard addition steps automatically and parallel to the fast determination by direct injection LC-ESI-MS/MS. Additionally, the automated sample handling increased the repeatability of multiple standard addition steps and reduced the possibility of error by manual sample preparation. The SAM was performed by analysis of the unspiked sample followed by consecutive standard addition steps (n = 4). This number of standard addition steps is often recommended in the literature. The SAM was completely conducted in one vial containing exact 1 mL sample. Each time 10 µL of the sample was used for the determination and was filled up with 10 µL spike solution again (added concentration each: 100 ng/L). Therefore, the sample volume was not changed and the matrix content varied insignificantly ≤ 4%. The advantages of this procedure compared to the often applied 1:1 dilution by spike solution and the use of one vial per added concentration were: no loss of sensitivity and increased free space on the sample rack, which enables a greater sample throughput. The integration and calculated relative standard deviation as well as the linearity were checked for each sample. The coefficient of correlation (R^2) was about 0.999 and the relative standard deviation (%RSD) less than 6%. Both were used as quality criterion for each sample. These values are comparable to other studies that used SAM [23, 43, 44].

4.4.4 Validation, method robustness and matrix effects

For the quantification by the SAM, no guidelines for method validation are available. Nevertheless, it is important to know some performance criteria in order to assess the behaviour of an analytical method. Therefore, the specificity, linearity, precision and sensitivity were investigated. The specificity of the analytical method was fulfilled by monitoring two SRM-transitions (quantifier-ion, qualifier-ion) and a predetermined retention time for each target analyte. The SAM requires a high repeatability and a wide linear operating range, due to the addition of stock solutions. The linearity of response was for all analytes investigated by reference solutions in pure water. For all substances, a good coefficient of correlation (R^2) of more than 0.999 was obtained. The precision values, expressed as relative standard deviations (%RSDs) were between 2 - 10% near the LOQ (30 ng/L), 1 – 7% in the middle concentration range (250 ng/L) and 1 – 5% at the higher end of the working range (800 ng/L) (repeated 10 times), respectively. The LOD and LOQ were determined according to the German norm DIN 32645 [45] using the calibration curve method. The calibration curve was established in the range between 5 – 130 ng/L with equidistant calibrators (n = 6). The LODs were in the range between 1 – 10 ng/L and LOQs were in the range between 3 – 35 ng/L. The validation results for all metabolites are summarised in Table 4-5.

Table 4-5: Results of validation: LOD, LOQ, coefficient of correlation (R^2), and recoveries as % relative standard deviation values (%RSDs) at different concentrations.

Analyte	LOD (ng/L)	LOQ (ng/L)	R^2	%RSD (30 ng/L, n = 10)	%RSDs (250 ng/L, n = 10)	%RSDs (800 ng/L, n = 10)
N,N-dimethylsulfamide	5	17	0.9994	3.2	2.8	2.1
desphenyl-chloridazon	2	9	0.9991	2.2	1.6	1.3
methyl-desphenyl-chloridazon	3	11	0.9990	1.9	1.3	0.6
2,6-dichlorbenzamide	3	9	0.9998	5.3	2.4	1.7
flufenacet-OA	4	14	0.9993	6.1	2.1	2.3
flufenacet-M2	8	29	0.9991	9.5	6.3	1.7
dimethenamid-P-M23	3	11	0.9999	2.1	1.5	1.6
dimethenamid-P-M27	4	15	0.9997	3.3	1.6	0.8
dimetachlor-CGA369873	2	6	0.9998	3.5	2.1	1.4
dimetachlor-CGA354742	3	11	0.9994	5.3	1.7	1.1
dimetachlor-CGA50266	2	8	0.9999	3.2	0.9	2.0
chlorthalonil-M5	5	17	0.9999	5.0	2.6	1.4
chlorthalonil-M12	3	9	0.9997	5.4	1.9	1.4
metazachlor-BH479-4	4	14	0.9998	6.1	2.3	1.8
metazachlor-BH479-8	7	27	0.9991	9.7	7.0	2.3
metazachlor-BH479-9	5	18	0.9993	6.1	2.8	2.2
metazachlor-BH479-11	1	5	0.9996	5.0	1.2	1.6
metazachlor-BH479-12	10	35	0.9990	8.7	3.9	4.4
S-metolachlor-CGA351916	3	9	0.9998	2.2	1.5	1.1
S-metolachlor-CGA-380168	3	10	0.9999	3.1	1.5	0.8
S-metolachlor-CGA368208	7	24	0.9998	9.5	2.1	1.6
S-metolachlor-CGA357704	1	4	0.9995	8.9	2.9	1.5
S-metolachlor-NOA413173	5	19	0.9996	3.3	2.6	0.9
metalaxyl-M-CGA62826	1	3	0.9999	1.5	0.8	0.8
metalaxyl-M-CGA108906	3	11	0.9997	2.5	2.4	1.7
quinmerac-BH518-2	7	26	0.9990	7.8	6.0	4.7
tritosulfuron-BH635-4	1	5	0.9998	2.8	2.5	1.9
dimoxystrobin-505-M08	4	14	0.9998	3.0	1.5	1.1
dimoxystrobin-505-M09	5	19	0.9998	4.0	0.7	0.7

To corroborate the robustness and accuracy of the SAM, various water samples (ten ground water, drinking water and surface water sampels) were spiked with spike solution (final spiked concentration 100 ng/L). The recoveries were determined by SAM and additionally external calibration to estimate the matrix effect. The percentage deviation of the known added amount was in the range of 90 to 110% for all analytes determined by SAM. The external calibration provided different results, with the early eluting metabolites (N,N-dimethylsulfamide, desphenyl-chloridazon,

methyl-desphenyl-chloridazon) showing recoveries up to 500%, and the remaining metabolites recoveries between 75 and 150% (shown in Figure 4-2). The high deviations of N,N-dimethylsulfamide, desphenyl-chloridazon, and methyl-desphenyl-chloridazon are consistent with previous studies that have investigated the influence of different salts as matrix constituents on these metabolites [11, 12].

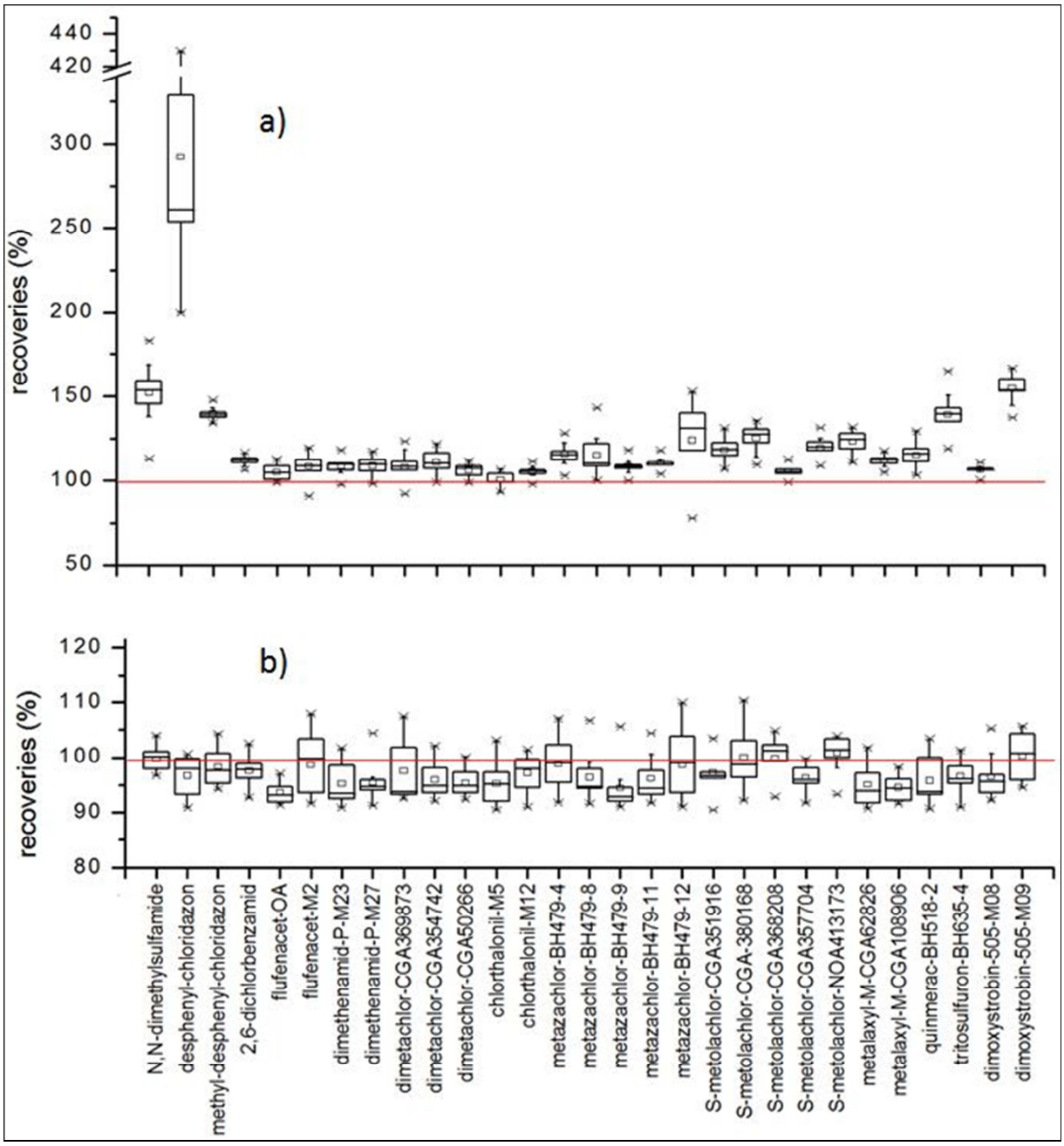

Figure 4-2: Box-Whisker plots showing recoveries of metabolites quantified by the external calibration method (a) and by SAM (b) in various environmental water samples (ten ground water, drinking water and surface water samples). The line in boxes represents the median, the boxes the 25 - 75% percentile, the whisker extends to the 95%-percentile of measured concentrations. The stars indicate the highest concentrations. The x-axis includes metabolites. The y-axis shows the recoveries in percent of known value of spiked concentration (100 ng/L). The red line indicates a 100% recovery.

4.4.5 Suitability for routine analysis

About 200 samples of drinking water, ground water, deep well water, surface water, and waste water were analysed with the presented method. Figure 4-3 to Figure 4-5 summarise the statistical data of the different water bodies in Box-Whisker plots. Approximately 94% of all samples contained one of the metabolites with a concentration of more than 30 ng/L. In more than 50% of these samples N,N-dimethylsulfamide, desphenyl-chloridazon, methyl-desphenyl-chloridazon, metazachlor-BH479-8 and S-metolachlor-CGA-380168 were detected. Flufenacet-M2, chlorthalonil-M12, metazachlor-BH479-4 and S-metolachlor-CGA351916 were found in more than 30% of these samples. Dimethenamid-P-M23, chlorthalonil-M5, metazachlor-BH479-9 and metazachlor-BH479-11 were not detected in any sample. These results can be correlated to the concentrations of the lysimeter tests (Table 4-1). The metabolites with higher concentrations in the lysimeter tests are in the majority of cases found in environmental samples. For drinking water samples the health related indication values (HRIV = 1 – 3 µg/L) for these metabolites was never exceeded. Only for desphenyl-chloridazon, the value (HRIV ≤ 3 µg/L) was exceeded in few ground water samples. Figure 4-3 shows the concentration of metabolites in waste water and surface water. The composition of metabolites in waste water is slightly different than in the other water bodies. In particular, the metabolites desphenyl-chloridazon and methyl-desphenyl-chloridazon are present in much lower concentrations, whereas the quinmerac metabolite mainly occurs in these samples. The origin of metabolites in waste water is frequently from municipal or private use, whereas the pesticide chloridazon is primarily and extensively used in agriculture as herbicide for sugar beets [12]. This explains the low concentrations in waste water and high concentrations especially in the ground water samples and also in other water bodies. The concentration of N,N-dimethylsulfamide in waste water is similar to other water bodies. The precursor of this metabolite is tolylfluanid that is still used in greenhouses but also in private areas as fungicide in wood preservatives or paints and for ornamental plants [46]. For the other metabolites due to the lower concentration in waste water, this cannot be a source for the entry into surface waters. Therefore, the major source of metabolites in surface water is probably surface runoff or drainage from agricultural fields.

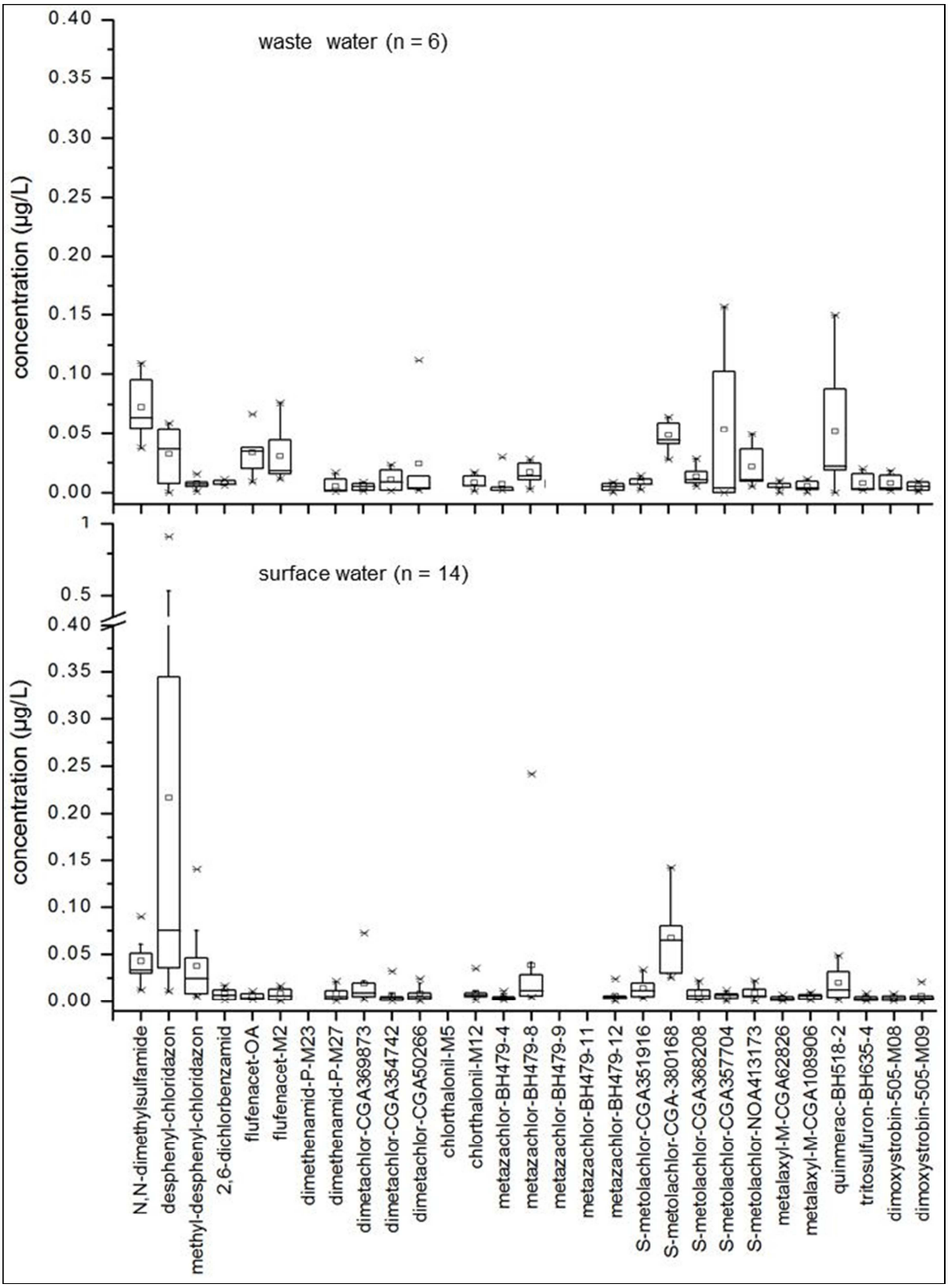

Figure 4-3: Concentration of metabolites in waste water (a) and surface water (b) as Box-Whisker plots. Y-axis shows the concentration in µg/L with an axis break at 0.40 µg/L. Above the axis break the concentration increases logarithmically, because for a better overview. The x-axis indicates the 29 investigated metabolites.

Figure 4-4 shows the concentration of metabolites in deep well water and ground water. In the ground water samples, not only the largest number of metabolites was detected, but also the highest metabolite concentrations were found. Even some deep well water samples, that should be free of contamination, contain metabolites in higher concentrations. These results are consistent with reported occurrence of metabolites in some natural mineral waters [12, 13].

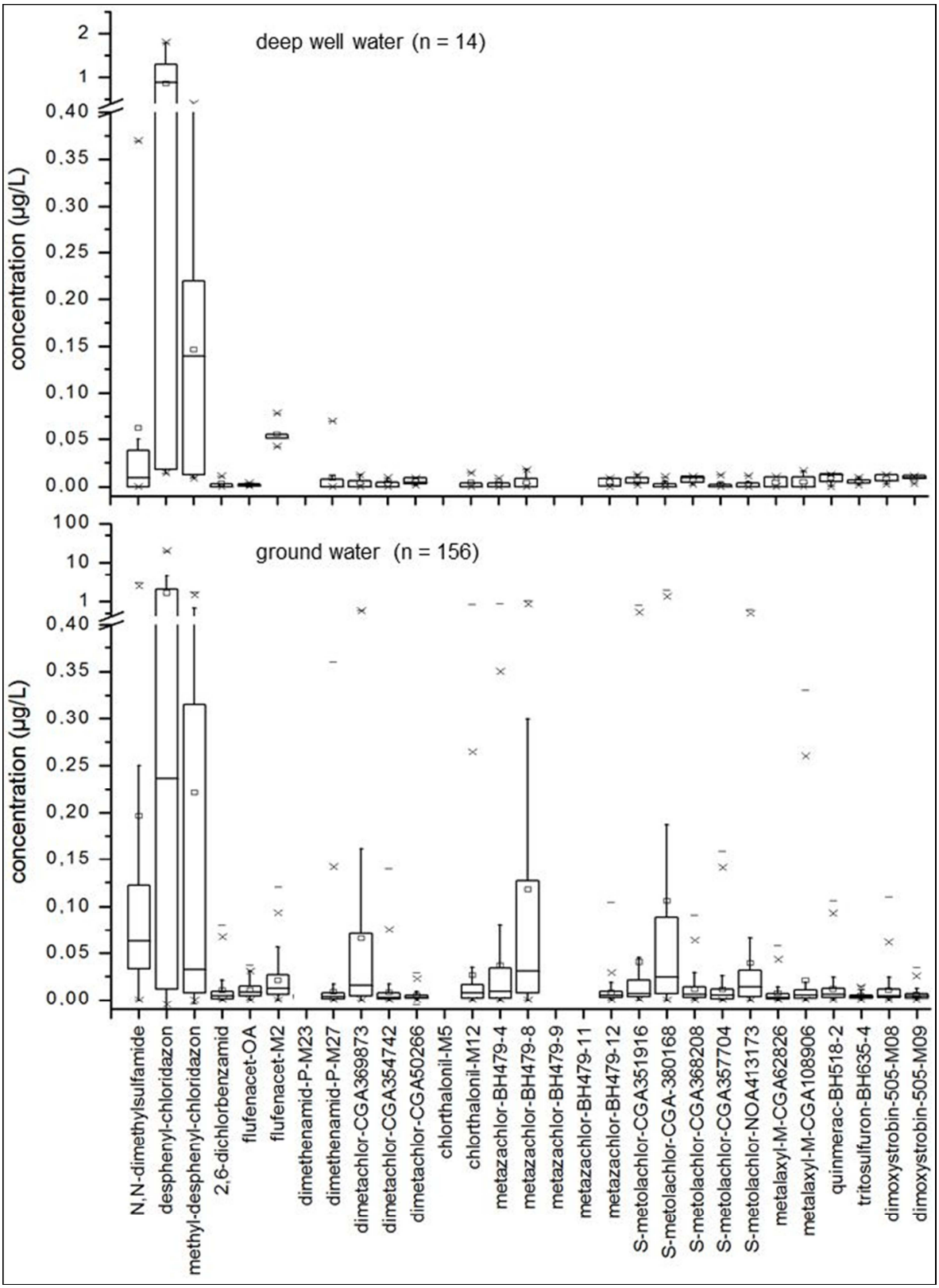

Figure 4-4: Concentration of metabolites in deep well water (c) and ground water (d) as Box-Whisker plots. Y-axis shows concentrations in µg/L and a logarithmically increase above the axis break (0.4 µg/L). X-axis shows the investigated metabolites.

In Figure 4-5 the concentration of metabolites in drinking water is shown. In drinking water, the previously mentioned metabolites with abundance of 50 and 30% in all water bodies were measured in very low concentrations. Only the most polar substances N,N-dimethylsulfamide, desphenyl-chloridazon and methyl-desphenyl-chloridazon showed elevated concentrations, because of the low removal efficiency with the usual drinking water treatment processes.

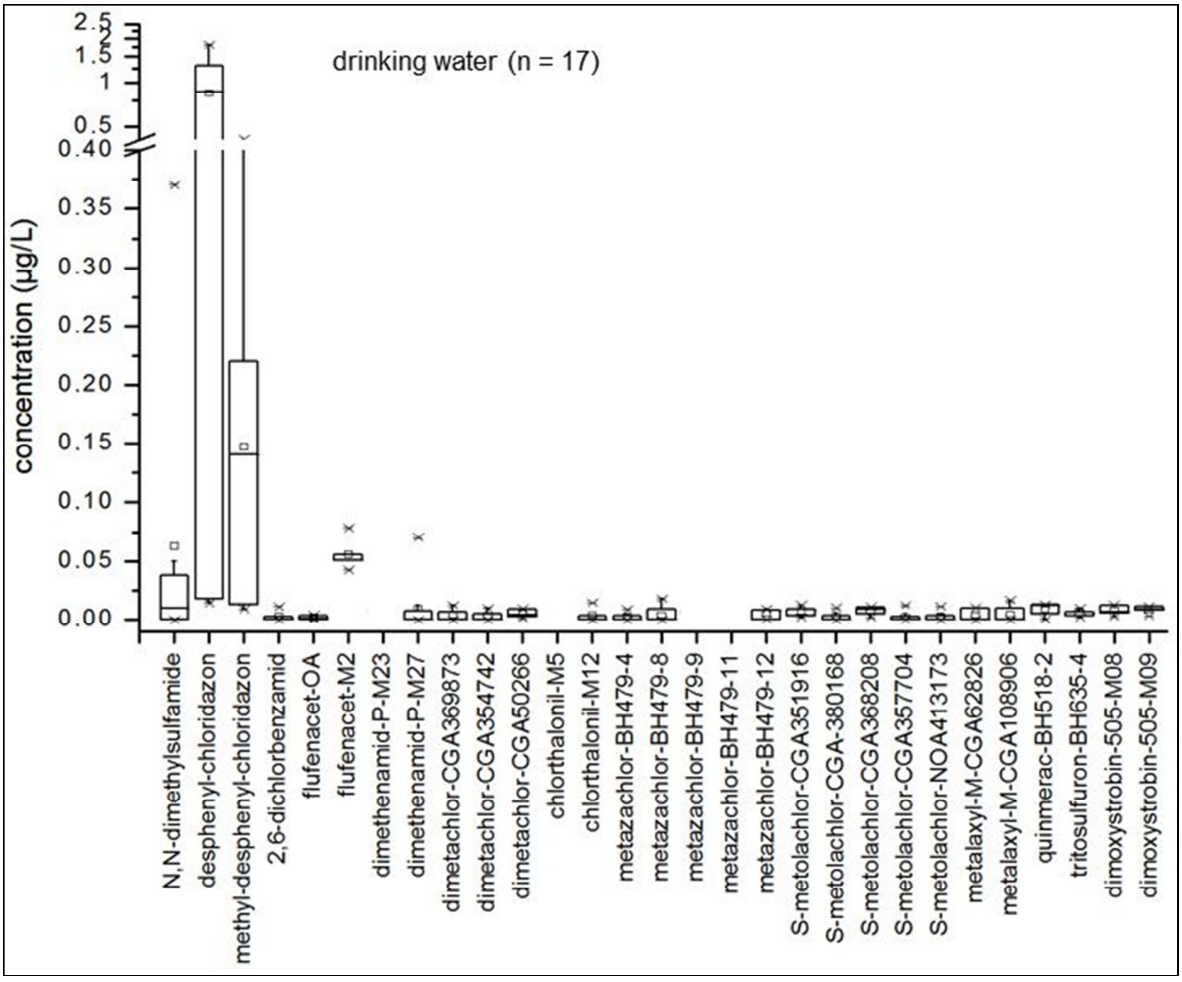

Figure 4-5: Concentration of metabolites in drinking water as Box-Whisker plots. The concentrations (µg/L) are shown on the y-axis with an axis break and increases logarithmically above the break (0.4 µg/L). X-axis shows the investigated metabolites.

4.5 Conclusion

The present study demonstrates a sensitive and reliable analytical method for the quantification of 29 polar metabolites of frequently used pesticides at trace levels in different water bodies in Germany. The SAM was successfully applied in routine analysis of environmental samples. The assumed drawbacks of this method (labour-intensive sample-handling, time-consuming multiple measurements and evaluation) could be eliminated by automating the manual steps, using a fast separation and detection system, and an automated evaluation process. Validation has shown that the SAM-based method is accurate and sensitive. Substantial matrix effects were observed in some real water samples precluding the use of external calibration. The reliability of this method was checked by recovery experiments in real water samples.

4.6 References

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Chapter 5. Degradation of the fungicide metabolite N,N-dimethylsulfamide (DMS) using hypochlorite

5.1 Abstract

N,N-dimethylsulfamide (DMS) – a metabolite of the fungicide tolylfluanid – has been detected for the first time in significant concentrations in ground water in different regions of Germany at the end of 2006. Because of the high polarity of DMS, it cannot be removed effectively by contemporary water treatment processes, e.g. bank filtration or use of activated carbon. Ozonation converts DMS into the highly potent carcinogenic N-nitrosodimethylamine, a compound with a health related indication values of 10 ng/L for drinking water. The behaviour of DMS during chlorination has not been studied, it is only known to degrade. In Germany, during the treatment of surface water to drinking water, chlorination is frequently used for so-called safety chlorination or transport chlorination, whereby oxidation transformation products can be formed. This work describes an approach for systematically identifying relevant chlorination transformation products of DMS in the laboratory scale. The solutions from different chlorination batch experiments were analysed by different chromatographic and mass spectrometric systems. Various approaches were used to detect and identify the chlorination transformation products. Dimethylamine and sulfate were detected as main chlorination transformation products. The estimation of relevance for drinking water treatment has shown that the formed dimethylamine and sulfate concentrations by chlorination were irrelevant in comparison to the occurrence in environment. Additionally, concentrations of dimethylamine formed during chlorination were well below toxicological and ecotoxicological thresholds.

5.2 Introduction

The application of numerous plant protection products (PPP) several times a year and over large areas lead to the infiltration of pesticide active ingredients (pesticides) into the aquifer by leaching and drainage processes [1]. Additionally, partial chemical or biological degradation of the pesticides on the surface or in the soil, cause the formation of usually more polar and persistent pesticide metabolites. During water purification processes, e.g. bank filtration or use of activated carbon, those polar pesticide metabolites frequently cannot be effectively removed. Additional risks may occur when these substances are chemically modified by oxidative processes like ozonation or chlorination within the drinking water purification, forming products of toxicological concern. An illustrative example is the case of N,N-dimethylsulfamide (DMS), which was found in significant concentrations in ground water from different regions in Southern Germany at the end of 2006 [2]. A concentration range between 0.1 and 1 µg/L was observed. Thereafter, nationwide concentrations of DMS were frequently determined in various groundwater and drinking water reservoirs. Own investigations (about 600 samples) have shown high concentrations of DMS, a 90-percentile value of 6.1 µg/L was found in ground water and up to 0.7 µg/L in several drinking water samples in the Rhine and Ruhr region of North Rhine-Westphalia (Germany) [3]. DMS is a biological metabolite of tolylfluanid, which has been used as a fungicide in fruit and wine culturing since approximately 1974. Because of its high polarity (log D = -0.2 calculated by Sparc online calculator V4.2, 2008) [4], DMS enters the aquifer and may not be effectively removed by current water treatment, like bank filtration or activated carbon adsorption [2]. Additionally, by the use of certain operational settings of an ozonation process during the water treatment, even small amounts of DMS can lead to the formation of carcinogenic N-nitrosodimethylamine (NDMA) [2]. On this basis, the outdoor application of tolylfluanid was banned in early 2007. Because of this particular behaviour of DMS during ozonation, this process has been studied extensively. However, although the main method used for the disinfection of drinking water is chlorination, this process was studied much less. It is only known that the disinfection by hypochlorite degrades DMS completely, however, it is unknown which chlorination transformation products (TPs) are formed [2]. The disinfection of drinking water in Germany is applied

according to §4 of the German drinking water ordinance (Trinkwasserverordnung, TrinkwV 2001, drinking water must be free of pathogens) [5]. Therefore, chlorination is applied to avoid contamination in the distribution system (safety or transport chlorination), or is required in the disinfection of drinking water originating from raw water obtained directly or indirectly from surface water. The chemicals used to disinfect drinking water are strong oxidizing agents, which can react with the naturally occurring DOC, bromide, and iodide but also anthropogenic trace substances such as pesticides or their metabolites [6]. However, for most of the known pesticide metabolites, no information about their behaviour during drinking water disinfection with regard to the formation of disinfection TPs is available. Based on this knowledge gap, the objectives of this work were: (i) non-target-screening for the detection of chlorination TPs from DMS, (ii) identification and structure elucidation by LC-MS/MS-experiments and measurement of accurate masses. An isotopically labeled D_6DMS and kinetics experiments were applied both for the clarification of detection and structure as well as for the determination of reaction pathways, and (iii) reference standard use for structure evaluation. Finally, the relevance of the formed chlorination TPs for drinking water treatment was assessed. The chlorination experiments of DMS were conducted in laboratory scale batch experiments. To identify the formed polar chlorination TPs from DMS, an ultra-performance liquid chromatography quadrupole tandem mass spectrometer (UPLC-MS/MS) was used. To characterise the structure, tandem mass spectrometric (MS/MS) experiments as well as the measurement of accurate masses with a LC-high-resolution(HR)-MS were applied.

5.3 Experimental

5.3.1 Reagents and materials

HPLC-MS/MS grade acetonitrile water and formic acid were obtained from Biosolve (Netherlands), N,N-dimethylsulfamide (DMS), purity 98%, was from ABCR GmbH & Co.KG (Germany). D_6-N,N-dimethylsulfamide (in following called D_6DMS), purity 99% was purchased from Campro Scientific (Germany). Ultrapure water was prepared by the water purification system Seralpur Pro 90 CN (Germany). Argon gas (5.0) for the collision cell was obtained from Air Liquide (Germany). Nitrogen for the mass spectrometric system was prepared by CMC instruments gas generator (Eschborn, Germany). Glass syringes (100, 250, 500 µL) were from Hamilton Bonaduz AG (Switzerland). Sodium hypochlorite solution (6-14% active chlorine) was obtained from Merck KGaA (Germany) and sodium thiosulfate pentahydrate 99% was purchased by Alfa Aesar Chemicals (USA). Ascorbic acid 99% and the standards of transformation products: dimethylamine solution 60% (v/v) aq., N,N-dimethylhydrazine 99%, sodium sulfate, sulfamic acid 99.3% were obtained from Sigma-Aldrich Co. (USA). Hand-held photometer SWAN Chematest 20 from SWAN Analytical Instruments (Switzerland) and solutions for the N,N-diethyl-p-phenylene diamine (DPD) colorimetric method were purchased from M. Hübers GmbH (Germany). Eppendorf pipet, Transferpette® was from Brand GmbH & Co. KG (Germany).

5.3.2 Preparation of the solutions and chlorination procedure

Chlorination solution was prepared from sodium hypochlorite stock solution, that contained 116 mg/L freely available chlorine and was diluted in two consecutive steps (116 mg/L to 11.6 mg/L to 1.16 mg/L). The freely available chlorine was determined by the portable chlorine photometer SWAN Chematest 20 using a commercial kit for the DPD colorimetric reaction. This instrument measures the absorbance and gives a value in mg/L Cl_2. The sodium thiosulfate solution (0.1 M) was prepared by dissolving 1.241 g $Na_2S_2O_3$ x 5 H_2O in 50 mL water. Equally, the ascorbic acid (0.1 M) solution was prepared by dissolving 0.8807 g ascorbic acid.

These solutions were used to prepare further dilutions of 0.001 M and 0.0001 M and were used as quencher for chlorination reactions.

For the standard solutions of DMS and D_6DMS (200 mg/L), approx. 5 mg of the substances were dissolved in a 25-mL volumetric glass flask, filled up with water and sonicated for 30 minutes. The different analytical standards of assumed chlorination transformation products were prepared by weighing (each 20 mg/L) and dissolving (50 mL) of the pure standard in acetonitrile. These solutions were used to prepare diluted spike or reference solutions, or for batch experiments, by diluting it in ultrapure water. All solutions were stored (4°C) in dark bottles. The aqueous reference solutions for the external calibration were prepared by diluting the stock solutions with pure water to yield a concentration range of 0.1 - 10 µg/L with nine calibration points. These solutions were also used to check the linearity and limit of quantification (LOQ).

All batch experiments were conducted at room temperature (22 - 24°C) and carried out with ultrapure water. Finally, the batch experiments for the total transformation rates were carried out also with drinking water. The glassware was exposed to sodium hypochlorite solution for at least 1 h and rinsed with water in order to avoid uncontrolled chlorine demand from the glass material. Afterwards, the vials for the samples were set up by adding 100 µL of the quencher solution and the sample volume from batch experiments (exactly 1 mL).

In the following, the general chlorination procedure is described. Depending on the particular requirements, the following experimental conditions varied: concentration of the target analyte and chlorine concentration (the specific conditions are shown in Figure 5-1).

For the non-target screening of the possible transformation products, the chlorination experiments were performed with a DMS concentration of 50 µg/L and free available chlorine of approximately 1.2 mg/L. The batch experiment was conducted in a 100-mL volumetric glassware flask filled up with ultrapure water to the final volume. For the reaction, 0.33 mL of DMS stock solution was added. The first sample was taken from this solution and marked as time 0 min. Afterwards, 1 mL of the 116 mg/L

chlorine solution was added (resulting in approximately 1.2 mg/L free Cl_2) to the flask, mixed thoroughly and the timer started. 1 mL of the samples was put in the vials by an Eppendorf pipet and was shaken. The time interval between each sample extraction was kept short during the first 10 minutes and extended as the reaction progressed. After approximately 24 h the reaction was stopped. In addition, one of the vials was filled with 1 mL of water containing 1.16 mg/L of free available chlorine without DMS and the other vial contained only DMS without chlorine reagent and was used as blank. The batch experiment for D_6DMS was conducted in the same way.

5.3.3 Non-target-screening

The non-target-screening of the samples from the batch experiments was performed with a Waters Acquity UPLC^{TM}-TQD (Milford, USA). The following chromatographic and mass spectrometric settings were used. An Acquity UPLC HSS T3 2.1 × 50 mm column with 1.8 µm particle size was used (Waters, Milford, USA). An acetonitrile/water mixture (2:98 v/v) with 0.01% formic acid was used as mobile phase with a constant flow rate of 360 µL/min. The columns were operated at 15°C by the use of an isocratic gradient for the first 2.5 min (isocratic elution). Afterwards, the content of acetonitrile was increased linearly up to 98% within 0.2 min and this ratio was kept constant for 0.3 min (cleaning phase). In the final step, starting conditions were re-established within 0.2 min and maintained for 0.8 min (conditioning phase). The injection volume was 10 µL, with a total runtime for the method of 4 min. The mass spectrometer operated in electrospray ionisation (ESI) positive and negative full-scan-mode. The mass range was set to m/z 20 – 250 and the dwell time was set to 0.5 second. The ESI–MS conditions were as follows: drying gas N_2 (1,000 L/h, 450°C); capillary voltage 0.800 kV; cone voltage varied from 10 - 40 V. The data were acquired in continuum mode and used as total ion chromatograms (TIC) for further evaluation processing. On the generated TICs, a component detection algorithm (CODA) was applied and the differences between the TICs are shown in 3D maps. Both are included in the master software MassLynx 4.1 (Waters, Milford, USA). These 3D maps and additionally the TICs were screened manually for new masses that appeared as a consequence of the chlorination process. The batch experiments for the kinetic experiments were conducted in the

same manner, however, with smaller concentrations of DMS, D_6DMS (5 µg/L) and the chlorination reagent (0.3 mg/L). In contrast to the previous procedure, a higher number of samples (n = 17) were taken over a period of 24 h. The determination of the reaction course was carried out in the selected-ion recording (SIR) by the same chromatographic settings. The majority of mass spectrometric settings were equal except: the specific masses were adapted from previously detected chlorination transformation products. The dwell time was 0.1 s per compound and the cone energy was 25 V due to a good intensity for all detected substances. The determination was subsequently conducted in ESI positive and negative mode. The areas from TICs were integrated and evaluated by TargetLynx software (included in MassLynx 4.1). The obtained areas were normalised at the greatest value ($area_i/area_{max}$) and the normalised values (0 - 1) were displayed in an Excel diagram on y-axis (Microsoft Office 2003, Microsoft Corporation, Redmond, USA) versus the reaction time (min) on the x-axis.

5.3.4 Identification, characterisation, verification and reaction path

The batch experiments were conducted equally, with 10 mg/L DMS or D_6DMS and 1.2 mg/L chlorine reagent. The samples, 17 in total, were measured by the previously developed SIR method to select only those samples for further investigations that included the previously detected compounds in high concentrations. Only these samples were measured by accurate mass and MS/MS-experiments.

Determination of the accurate mass and proposing an empirical formula was achieved by using a high performance liquid chromatography, quadrupole time-of-flight mass spectrometer HPLC-Q-TOF-MS (Series 1100 / 6520, Agilent Technologies, Santa Clara, CA). A Zorbax Eclipse XDB-C18, 1.8 µm, 2.1 x 100 mm column (Agilent Technologies, Waldbronn, Germany) with a flow rate of 0.6 mL/min at a temperature of 25°C was used for chromatography. The mobile phase contained water (A) and methanol (B) both with 0.1% (v/v) formic acid. The gradient was isocratic 1% B to 4 min, than increased linearly to 95% B at 8 min and was continued to 10.5 min. The Q-TOF-MS ran was conducted successively in positive and negative ESI mode with a capillary voltage of 3.6 kV; N_2 (10 L/min; 350°C) was used as drying gas, the spraying gas pressure was operated at 3.45 bar. In MS-scan mode, data

were acquired at a scan rate of 1 spectrum per second and a scan range from 30 to 1050 m/z. The calibration was performed with an Agilent standard calibration mixture for Q-TOF-MS systems. Acquired data were evaluated with the MassHunter Workstation (Agilent Technologies, Santa Clara, CA). This software includes an empirical formula generator tool. The experimental masses and isotope patterns were compared to theoretical monoisotopic masses. The resulting suggestions of empiric formulas were in an absolute score that can vary from 0 to 100 (100% accordance).

The tandem mass spectrometric MS/MS-experiments for the investigation of structure information, verification and quantification were also carried out by Waters Acquity UPLC^{TM}-TQD. The previously chosen samples from the batch experiments for accurate mass determination were diluted 1:100 with ultrapure water for the following investigations. The separation was conducted by a longer HSS T3 2.1×100 mm column. The gradient and column temperature conditions were compared to the previous experiments by this equipment. The mass spectrometer operated in ESI positive and negative mode and acquired data in MS/MS-scan mode for structural characterisation. The ESI-MS conditions were equal to the previous experiment. The additional MS/MS conditions were: argon as a collision gas at a flow of 0.15 mL/min and a collision energy ramp from 10 to 40 eV. For the in-source fragmentation experiment a higher cone energy (120 V) was used. The first quadrupole was used in static mode for separating the specific fragment mass (m/z 80) and the third quadrupole was used in dynamic mode for daughter scan. The acquired TICs were evaluated for specific previously detected peaks (double retention times compared to a 50 mm column) and for this retention time, the MS/MS spectra were extracted.

The verification was carried out in selected-reaction monitoring (SRM). The separation conditions were equal to the previous SIR method. For the tuning of MS settings, analytical standards with the assumed chlorination transformation products were used at a concentration of 250 µg/L. By Auto-Tune-Wizard the product ions, cone voltage, CID energy were optimised and up to four precursor ions for each compound were acquired. By comparing the extracted ion chromatograms (EIC), two

of the four precursor ions with the highest signal to noise ratio were chosen for further investigations. The chlorination batch experiments were conducted in the same way for the kinetic investigations ($c(OCl^-)$ = 0.3 mg/L; n = 17), however with smaller concentrations of DMS (3 µg/L) over a period of 24 h. In addition, the experiments were conducted three times with samples directly from a drinking water treatment effluent. At the same time, the samples from the batch experiments and reference solutions of purchased standards were defined. The EIC of the samples from the batch experiments and reference solutions were compared and are illustrated in Figure 5-7 and Figure 5-8. Further, these raw data sets were used for the quantification of the total conversions rate. The responses of the nine analytical standards were used for external calibration and to estimate the LOQ. The LOQ was estimated by signal to noise procedure (9:1) on EIC. For the integration, evaluation and quantification the TargetLynx software was used.

5.4 Results and Discussion

The detection and characterisation of transformation products (TP) from DMS during chlorination by LC-MS required higher analyte concentrations because of the lack of intensity of MS modes used and MS equipment used. For non-target screening and structural clarification, the dynamic MS-scan and MS/MS-scan mode are required. Due to lack of intensity of these modes, significantly higher concentrations of target analytes are necessary. This applies particularly to high-resolution devices such as time-of-flight (TOF) or Orbitrap^{TM}. In addition, the high-resolution devices are less sensitive especially in the case of small molecules (m/z < 150). Therefore, the substances are often enriched if possible or used in higher concentrations in laboratory experiments. Due to the high polarity of DMS and commonly increasing polarity of the formed chlorination TPs, enrichment methods such as solid-phase extraction or liquid-liquid extraction may not be used due to lack of efficiency. High concentrations of DMS could influence the reaction of the chlorination experiment. Therefore, the samples of the batch experiments are injected directly into LC-MS system without sample preparation. The concentrations of DMS were adjusted in dependence of the experiments performed and LC-MS equipment used. The investigation of DMS degradation during chlorination experiments was performed on two LC-MS systems. A more sensitive Water Acquity UPLC^{TM}-TQD was used for non-target-screening, for the elucidation of structures by MS/MS-experiments, and for the evaluation of structures by analytical standards of the chlorination TPs formed. An Agilent HPLC-Q-TOF was used to generate empirical formulas. The specific MS modes of the corresponding experiment and the used work flow are illustrated in Figure 5-1. To assist the identification and structure elucidation, an isotope-labeled D_6DMS analytical standard was used, which was previously purchased for the development of an analytical method for the quantification of DMS [3].

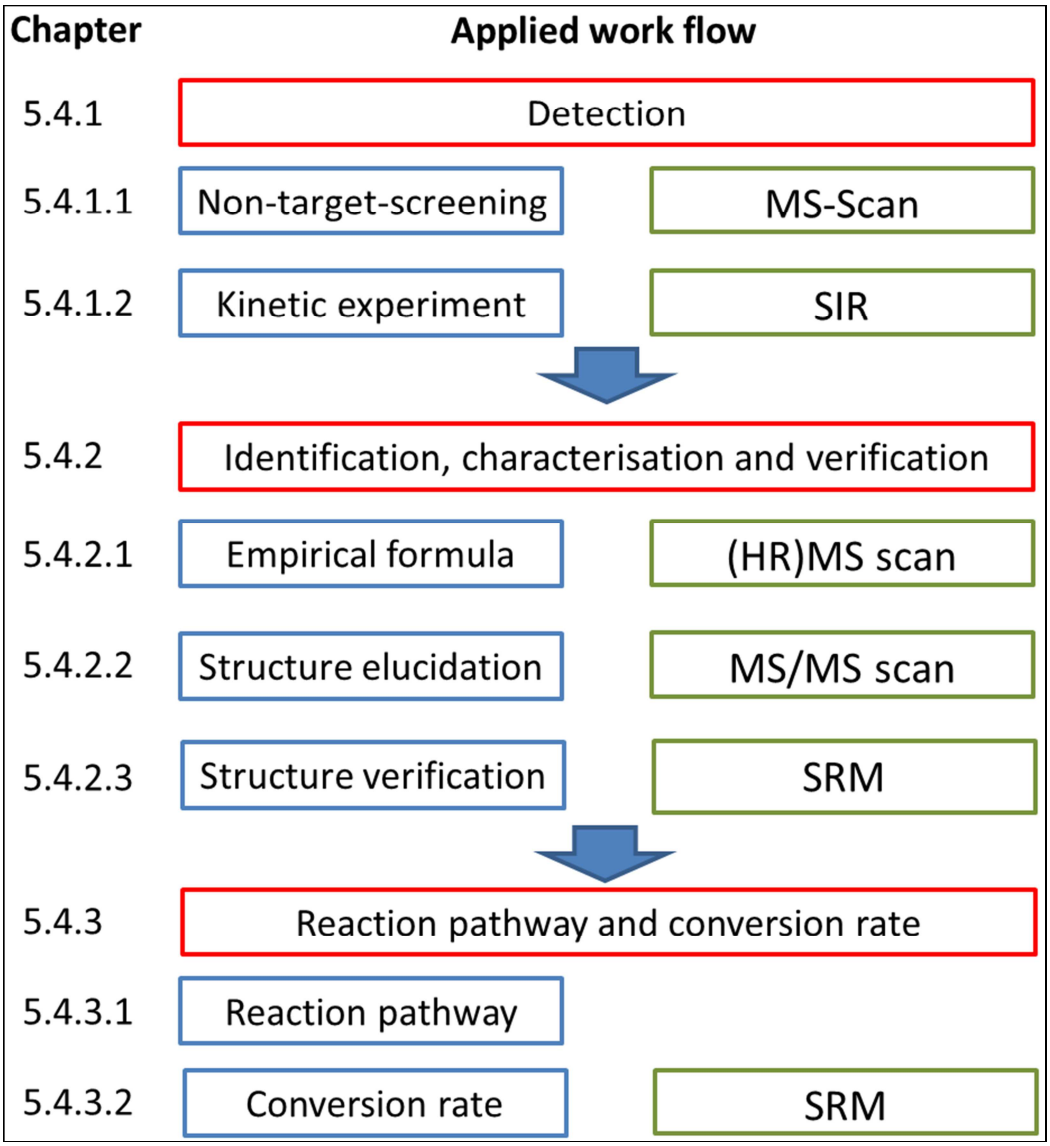


Figure 5-1: Illustration of the applied work flow including: chapter numbers, conducted investigates and applied modes of MS equipment.

5.4.1 Detection

5.4.1.1 Non-target-screening

Previous investigations have shown that the average concentration of DMS in drinking water is 0.7 ug/L. Due to the limitation of sensitivity of the MS instrument used during non-target-screening, the batch experiments were conducted with a concentration of 50 µg/L. The isotope-labeled D_6DMS standard was used with the same concentration. The free chlorine concentration and the batch experiment reaction time are regulated by the German drinking water ordinance

(Trinkwasserverordnung, TrinkwV 2001), which allows a dosage of chlorine or chlorine compounds (sodium and calcium hypochlorite) up to maximal 1.2 mg/L of freely available chlorine. After a reaction time of at least 30 minutes, the residual concentration should be maximal 0.3 mg/L for 24 h. The batch experiments were carried out with similar concentrations and reaction time (depending on the experimental conditions). The chromatographic conditions were taken from a previous study [3] that was developed for the quantification of DMS using a column with a low carbon loading, and therefore appropriate for the separation of polar compounds. The column temperature was held at 25°C to prevent further degradation of the chlorination TPs. The use of a UPLC-equipment in combination with a short column (2.1 diameter and 1.7 µm particle size) leads to narrow and high peaks that have a high signal to noise ratio [7, 8]. The other advantage of using small particles is the high chromatographic separation efficiency, which enables a short runtime in less than 5 min [3, 9]. The chosen chromatography techniques permit easy peak picking in the extracted ion chromatograms (EIC) of formed chlorination TPs and lead to small raw-data files that need to be evaluated manually. For the screening of formed polar chlorination TPs from DMS and D_6DMS, ESI in positive and negative mode were used to detect acidic, basic and neutral compounds. The MS scan mode from m/z 20 - 250 and 0.5 s dwell time was used, because a small mass range and high dwell time increase the sensitivity during acquisition by a quadrupole mass spectrometer. For selection of the mass range, it was assumed that the formed TPs could not exceed the twofold mass range. The evaluation was performed by comparing the respective TICs with each other manually. To improve the identification, an algorithm to reduce the background noise was applied. This algorithm worked as follows: it removes mass chromatograms that represent background noise from the dataset by comparing each raw mass chromatogram to a smoothed, standardised mass chromatogram. Figure 5-2 shows an example of the difference in the TICs of the samples from chlorination experiments at different times without (a) and with the application of the algorithm (b). The filtered TICs were imaged in a 3D map diagram. The m/z and the retention are displayed on the x- and y-axis, while the z-axis indicates the intensity of the peaks in different colours. By using 3D maps, the TIC was displayed more clearly and differences could be detected more easily. The display of the 3D map can be modified between high

intense mode (peaks in the intensity range of noise are displayed) and low intense mode, where exclusively highlighted intense peaks are visible. Because of the fast chromatographic separation and the limited mass range, the raw data could be compressed for manual evaluation. This led to significant time savings in the evaluation of the TICs among themselves.

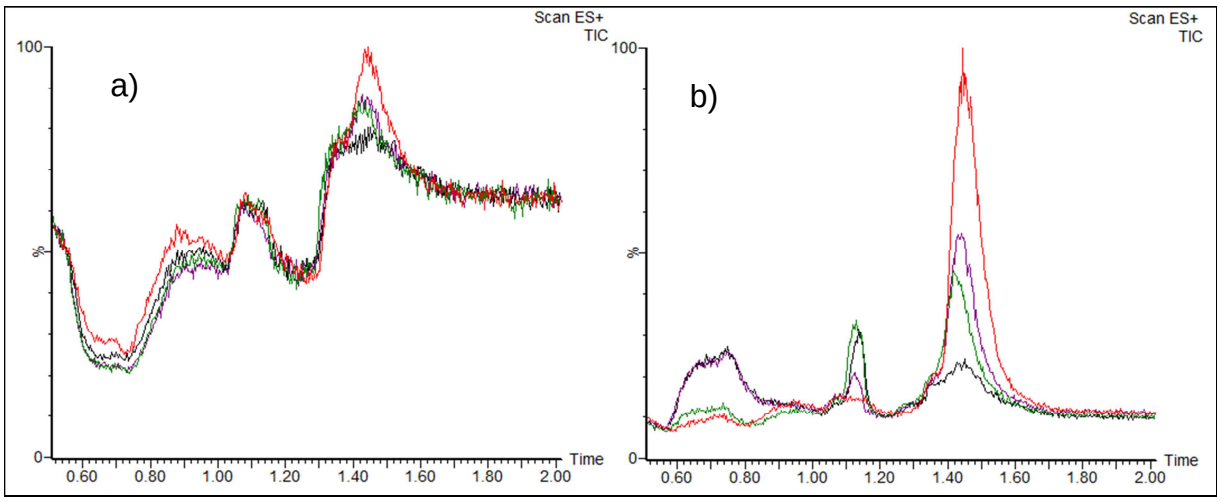


Figure 5-2: TIC of the samples from chlorination experiments at different reaction times (red t = 0 min; green t = 0.5 min; purple t = 30 min and black t = 24 h) without (a) and with the application of the algorithm (b) to improve the signal to noise ratio.

The non-target-screening evaluation of the batch experiments based on the 3D map diagrams included the processed data of the samples from chlorination experiments at different times (0 min, 0.5 min, 30 min, 5 h and 24 h) and two blanks (water + DMS + $S_2O_3^-$ or ascorbic acid without OCl^- and water + OCl^- + $S_2O_3^-$ or ascorbic acid without DMS). As described in the experimental section, two different quenchers were used to stop the reaction process. The 3D map diagrams at reaction times of 0 min, 0.5 min, and 24 h show the main differences in chlorination experiments and are illustrated in Figure 5-3 and Figure 5-4. The differences between the individual samples and the two blanks indicate the formation of chlorination TPs. Figure 5-3 and Figure 5-4 show the 3D map diagrams for DMS and D_6DMS in positive (Figure 5-3) and negative (Figure 5-4) ESI mode. It can be seen that the degradation pathways of DMS and D_6DMS led to the formation of identical TPs with the difference, that some TPs of D_6DMS include mass differences of 6 Da. The following designation of detected $[M+H]^+$ or $[M-H]^-$ masses in positive or negative ESI mode contain in the following only the molecular masses, and were applied to DMS and D_6DMS in the

same manner (M45, M58, M60, M97, M98, M107, M125 and M140a/b). Therefore, the M45, M58, M60, M107, M140a were formed in positive and M97, M98, M125, M140b in negative ESI mode.

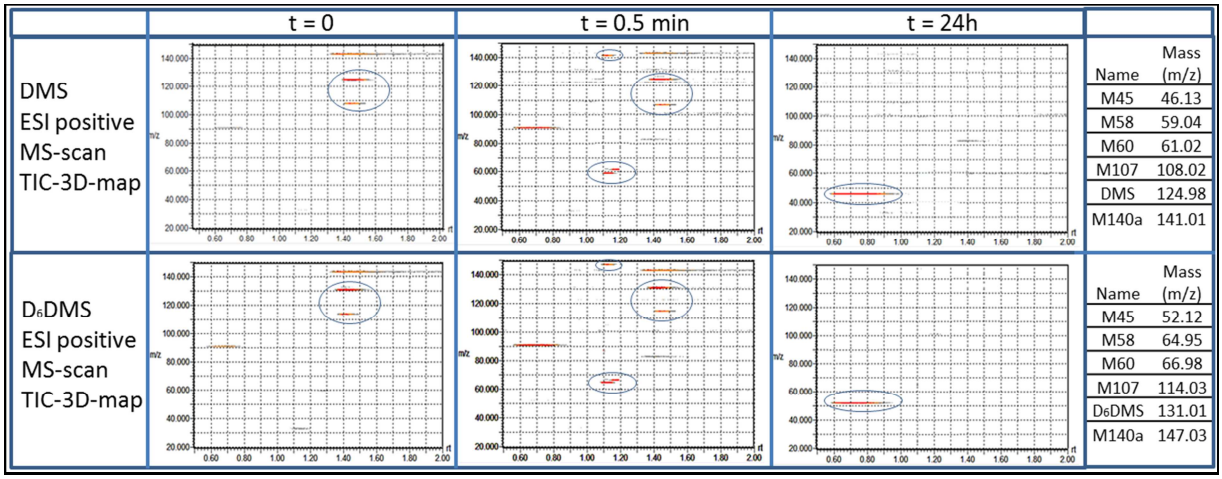


Figure 5-3: Selected 3D maps of detected TPs (only the interesting mass range m/z 20 - 150 and retention time range 0.4 - 2 min is displayed) in ESI positive mode of different batch experiments at several reaction times (t = 0 min, 0.5 min and 24 h). As quencher reagent, ascorbic acid was used. A high intense level was chosen to reduce noise and to focus only on highest peaks.

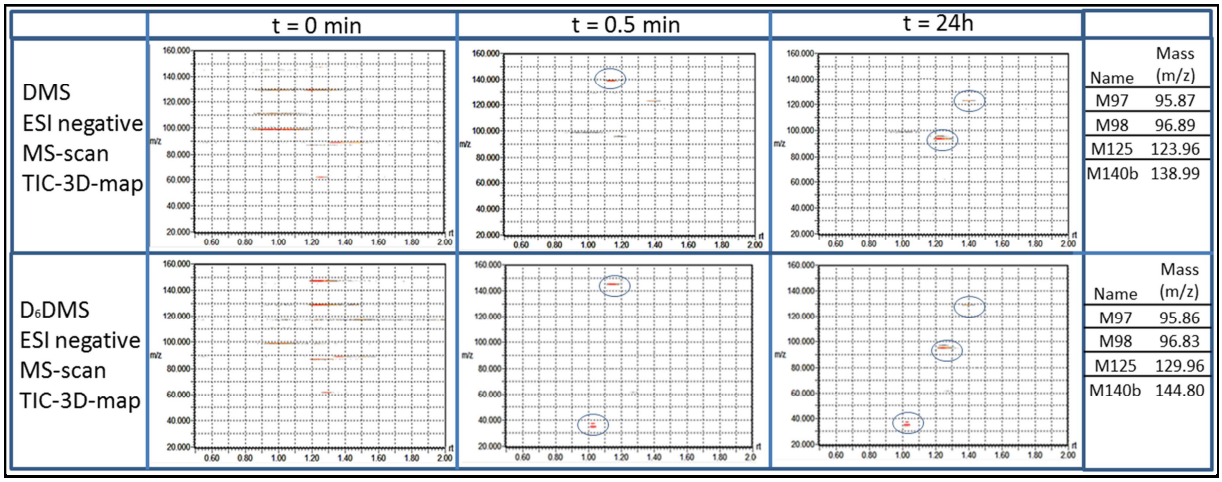


Figure 5-4: Selected 3D maps of detected TPs (only the interesting mass range m/z 20 - 150 and retention time range 0.4 - 2 min is displayed) in ESI negative mode of different batch experiments at several reaction times (t = 0 min, 0.5 min, and 24 h). As quencher reagent, ascorbic acid was used. A high intense level was chosen to reduce noise and to focus only on highest peaks.

The 3D maps show a complete degradation of DMS after 24 h. In the blank of DMS and at time t = 0 and 0.5 min, the TP M107 with the equal chromatographic behaviour as DMS (m/z 108) and D_6DMS (m/z 114) was detected. M107 does not

exist in the blank without DMS. Due to the similar retention times, it was assumed that a decomposition of DMS happened during the ionisation process rather than in solution. During the degradation of DMS, within a very short time (t = 0.5 min) some intermediates (M58, M60, M97 and M140a/b) were formed. After 24 h, the intermediates were completely degraded and the stable degradation products M45, M97, and M125 were formed. In addition to the chlorination TPs of DMS, other substances from the chlorination and quencher reagents themselves (m/z 35, 37, 97 in ESI negative mode and m/z 177, 181, 189, 191 in ESI positive mode) were detected in the samples after chlorination as well as in the blanks. It was assumed that the masses m/z 35 and 37 show the formation of chloride ions. The masses m/z 97 and 177 are from the quencher reagent (thiosulfate and ascorbic acid), because these were formed in the blank sample not containing DMS. The masses m/z 181, 189 and 191 however, formed in both samples and blanks could not be assigned to any of the used reagents. These masses are no longer considered. The mass m/z 97 in the negative ESI mode was formed by the use of thiosulfate as a quencher in the blank solution without DMS, but not with the used ascorbic acid as a quencher. Via redox reaction, HSO_4^- (97 m/z) was formed and detected from thiosulfate. HSO_4^- was also detected during DMS degradation (97 m/z or M98). The additional formation of HSO_4^- from thiosulfate can affect the reaction of DMS. Therefore, in further batch experiments only ascorbic acid as quencher was used. All hitherto detected relevant chlorination TPs are shown in Table 5-1.

Table 5-1: Detected masses of chlorination TPs from DMS and D_6DMS in positive and negative ESI mode. The table contains the designation of formed TPs. In addition, the results of the nitrogen rule are described for all detected substances.

ESI positive mode			
DMS		D_6DMS	
Name	Mass (m/z)	Mass (m/z)	Nitrogen rule
M45	46,13	52,12	0 or 1
M58	59,04	64,95	0 or 2
M60	61,02	66,98	0 or 2
M107	108,02	114,03	0 or 1
DMS/D_6DMS	124,98	131,01	0 or 2
M140a	141,01	147,03	0 or 2

ESI negative mode			
DMS		D_6DMS	
Name	Mass (m/z)	Mass (m/z)	Nitrogen rule
M97	95,87	95,86	0 or 1
M98	96,89	96,83	0 or 2
M125	123,96	129,96	0 or 1
M140b	138,9	144,8	0 or 2

5.4.1.2 Kinetic experiment

Another method for the detection and assignment of chlorination TPs is the monitoring of the reaction process (kinetic experiments). The illustration of time courses of the chlorination reaction of all hitherto detected compounds (Figure 5-5 and Figure 5-6) allows visual estimation of their degradation behaviour and distinction between intermediates and main products. For this purpose, over a course of the reaction of 24 h, a large number of samples were examined (n = 17). Monitoring intervals at the beginning of the reaction were short and increased over time during the chlorination reaction. All previously detected relevant TPs from non-target-screenings were examined. The batch experiments were carried out under the same conditions as above, but with a lower concentration of DMS and D_6DMS (each 5 µg/L), and a chlorine concentration of 0.3 mg/L. The determination was performed in the more sensitive SIR mode. The cone voltage was varied by the use of a cone-ramp (10 - 40 V) during the acquisition, and the chromatographic settings remained unchanged. From Figure 5-5 and Figure 5-6, it can be seen that the TPs (M45, M58, M60, M107, M125, M140a/b) with a mass difference of 6 Da between DMS and D_6DMS have a similar reaction pathway. This is also true for the TPs M97 and M98 without differences in the masses between DMS and D_6DMS. The TPs from DMS and D_6DMS previously identified as relevant, were confirmed by similar reaction pathways (Figure 5-5 and Figure 5-6). In addition, it was confirmed that M107 was not a TP of DMS. Because of the identical time course of M107 and DMS displayed

in Figure 5-5, it was assumed that M107 was formed from DMS by loosing an amine group during the electro-spray ionisation process. By the close examination of the reaction process, it can be assumed that a direct degradation of DMS, after the addition of the oxidising agent took place and formed intermediates (M58, M60 and M140a/b), which by further chlorination process led to the main degradation products (M46 and M98) after about 20 min. Also a small amount of the same main chlorination TPs are formed immediately after the degradation of DMS (M46, M97, M98 and M125).

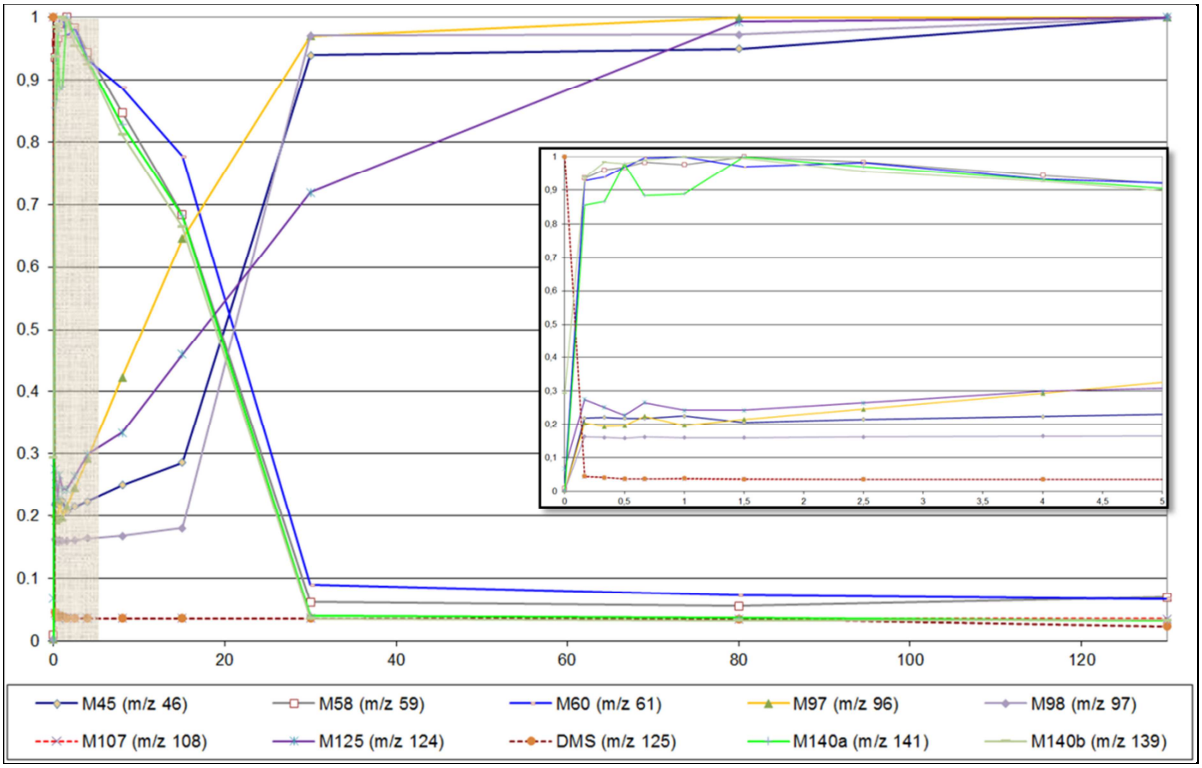


Figure 5-5: Illustration of the reaction time course of DMS by the chlorination and the formation of chlorination TPs. X-axis shows the reaction time of batch experiment and y-axis displays the normalised area values ($area_i/area_{max}$). The graph was cut at 130 minutes, since after this reaction time no further changes were observed. The inset graph shows the first 5 minutes of the total reaction.

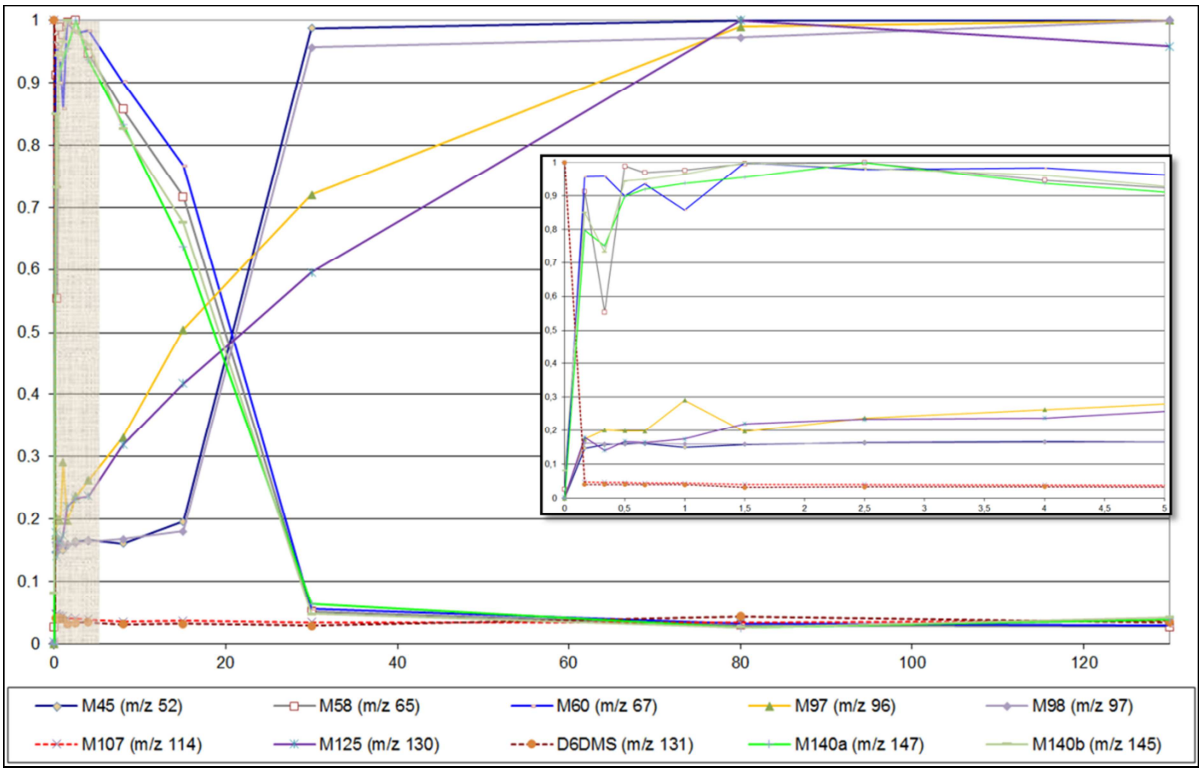


Figure 5-6: Illustration of the reaction time course of D_6DMS by the chlorination and the formation of chlorination TPs. X-axis shows the reaction time of batch experiment and y-axis displays the normalised area values ($area_i/area_{max}$). The graph was cut at 130 minutes, since after this reaction time no further changes were observed. The inset graph shows the first 5 minutes of the total reaction.

5.4.2 Identification, characterisation and verification

5.4.2.1 Determination of empirical formula

In order to characterise the possible structure of formed chlorination TPs, accurate masses were determined with an Agilent LC-Q-TOF mass spectrometer and Waters tandem mass spectrometer, which uses LC-MS/MS experiments. The further determination includes the chlorination TPs of DMS as well as D_6DMS previously classified as relevant. Because of the low sensitivity of the high resolution device, the batch experiments were performed again with a higher DMS and D_6DMS concentration of 10 mg/L and were diluted 1:100 for the MS/MS experiments. The batch experiments contained 17 samples again, which were investigated with the existing SIR detection method for selecting exclusively the samples, which contained the relevant TPs in sufficiently high concentrations for further investigations. For all previously detected TPs from DMS and D_6DMS, the high resolution mass

spectrometry could be used for the generation of an empirical formula. The formation of the empirical formula was limited to the following elements: C, H, D, S, O, N and Cl and included both the accurate mass and the isotope ratios. The proposed empirical formulas are included in Table 5-2. The empirical formulas were selected from different proposals with a score greater than 97% and where the differences between the experimental masses and theoretical monoisotopic masses were less than 5 ppm. For the same TPs of DMS and D_6DMS, the difference of 6 Da could be detected. Thus, these TPs (M45, M60, M125 and M140a/b) include the two methyl groups of the dimethylamine group from DMS. The nitrogen and double bond rule are generally useful to predict the structure of a substance. For M45, M97 and M125 zero or one nitrogen atom and for M58, M60, M140a/b zero or two nitrogen atoms were predicted. The double bond rule predicted one double bond for M58.

Table 5-2: Experimental and theoretical data from the determination of accurate masses of chlorination TPs from DMS and D_6DMS in positive and negative ESI mode. Including also experimental nominal masses, empirical formula and results from double bond rule.

ESI positive mode

DMS					D_6DMS				
Name	Exp. nominal mass [M+H]+ (m/z)	Exp. Mass [M+H]+ (m/z)	Theort. Monoisotopic mass [M+H]+ (m/z)	Empirical formula	Exp. nominal mass [M+H]+ (m/z)	Exp. Mass [M+H]+ (m/z)	Theor. Monoisotopic mass [M+H]+ (m/z)	Empirical formula	Double bond rule (n= double bonds)
M45	46,13	46,0657	46,0657	C2H7N	52,12	52,1035	52,1033	C2HD6N	0
M58	59,04	59,0609	59,0609	C2H6N2	64,95	65,0987	65,0986	C2D6N2	1
M60	61,02	61,0768	61,0766	C2H8N2	66,98	67,1145	67,1142	C2H2D6N2	0
DMS/D_6DMS	108,02	125,0388	125,0385	C2H8N2O2S	131,01	131,0764	131,0761	C2H2D6N2O2S	0
M140a	141,01	141,0332	141,0334	C2H8N2O3S	147,03	147,0713	147,0710	C2H2D6N2O3S	0

ESI negative mode

DMS					D_6DMS				
Name	Exp. nominal mass [M-H]- (m/z)	Exp. Mass [M-H]- (m/z)	Theor. Monoisotopic mass [M-H]- (m/z)	Empirical formula	Exp. nominal mass [M-H]- (m/z)	Exp. Mass [M-H]- (m/z)	Theor. Monoisotopic mass [M-H]- (m/z)	Empirical formula	Double bond rule (n= double bonds)
M97	95,87	95,9753	95,9755	H2NO3S	95,86	95,9753	95,9755	H2NO3S	
M98	96,89	96,9595	96,9596	H2O4S	96,83	96,9595	96,9596	H2O4S	
M125	123,96	124,0064	124,0068	C2H7NO3S	129,96	130,0446	130,0445	C2HD6NO3S	0
M140b	138,99	139,0180	139,0177	C2H8N2O3S	144,8	145,0557	145,0554	C2H2D6N2O3S	0

5.4.2.2 Structure elucidation

The MS/MS experiments were carried out by UPLC^{TM}-TQD in MS/MS-scan mode. The CID energy varied from 10 – 40 eV. A longer separation column (10 cm) was used under the same chromatographic conditions. This led to broad peaks and allowed to increase the existing scan rate per peak, and therefore the signal response intensity. The MS/MS spectra of the chlorination TPs and for DMS and D_6DMS are shown in Figure 5-7 and Figure 5-8.

The MS/MS spectra of the chlorination TPs can be interpreted as follows: for M45 the charged precursor ions m/z 30, 31 (DMS) and 32, 34 (D_6DMS) were detected. These fragments were methylamine with double bond (m/z 30 CH_4N^+ or m/z 32 CH_2DN^+) and single bond (m/z 31 $CH_5N^{+\cdot}$, m/z 34 $CH_2D_3N^{+\cdot}$). The uncharged fragments are m/z 15 (CH_3) or m/z 18 (CD_3). Because of the fragment patterns observed for M45, the compound dimethylamine (DMA) was assumed. The LC-MS/MS spectrum of dimethylamine (MassBank Record: KO002758) from an open-source data base (MassBank) [10] was compared with the generated MS/MS spectrum. The mass spectra were equal.

For M58 two nitrogen molecules (obtained from molecular mass) and one double bond (obtained from empirical formula) were predicted. The charged product ion m/z 29 was equal for DMS and D_6DMS and the mass of the uncharged molecule of this fragment predicted two nitrogen molecules. For these fragments, a hydrazine molecule (HN_2^+) was assumed. The product ions m/z 43, 44 (DMS) and m/z 46, 47 (D_6DMS) with a difference of 3 Da indicated a monomethyl molecule. These fragments were the monomethylhydrazin ($CH_3N_2^+$ and $CH_4N_2^{+\cdot}$) and the interpretation of all fragments led to the assumption of the precursor ion 1,2-dimethylhydrazin (symmetrical-DMH or SDMH). However, for M60 the m/z 44, 45 (DMS) and 50, 51 (D_6DMS) a mass difference of 6 Da each was observed. The double bond rule predicted a single bond. Therefore, 1,1-dimethylhydrazine (unsymmetrical-DMH or UDMH) was assumed for M60. The chlorination TPs M97, M98, M125 and M140a/b show only one charged product ion (m/z 80), both for DMS and for D_6DMS. By in-source fragmentation and subsequent fragmentation in the collision cell further fragmentations similar to MS^3 were performed. The M98 was used for in-source fragmentation experiments. The m/z 80 was detected by in-source fragmentation with 120 V cone energy and the m/z 64 was detected after the collision cell experiment. For the charged m/z 64 fragment SO_2^- and for m/z 80 fragment SO_3^- was assumed, respectively. For M97 and M98, no differences between precursor ions of DMS and D_6DMS were detected. The difference of 17 Da for the uncharged fragment of M98 is typical for OH or NH_3 groups. Therefore, it is difficult to make a clear assignment for both chlorination TPs of M97 and M98 from the MS/MS spectra. To predict a structure of these chlorination TPs only the empirical formulas were used. For the TP M97 sulfamic acid was assumed and for M98 sulfuric acid (sulfuric acid is possibly a species that occurs during the ionisation process, in the batch experiments sulfate is more probable so that this species is mentioned in the following sulfate). The precursor ions of M125, 140a/b shows a mass difference of 6 Da by DMS and D_6DMS. The mass difference between the precursor ion and product ion was 45 Da (M125) and 60 Da (M140a/b). This corresponds to the previously mentioned chlorination TPs M45 and M60. Therefore, for the TP M125 dimethylsulfamic acid and for M140a/b 2,2-dimethylhydrazinesulfonic acid was assumed.

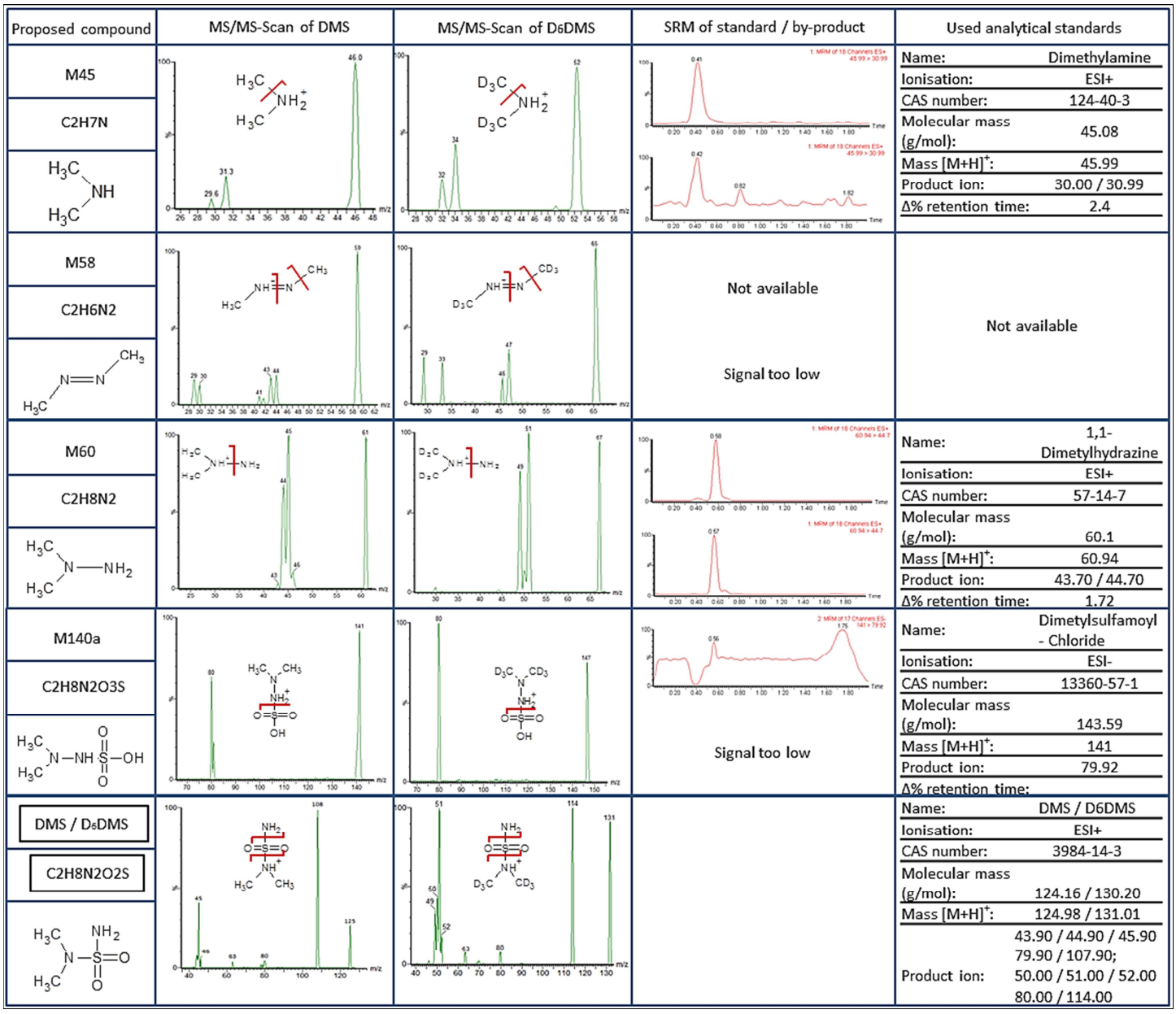


Figure 5-7: Assumed empirical formulas, structures, and MS/MS spectra of chlorination TPs in ESI positive mode of DMS (left) and D_6DMS (right). In column four, the SRM chromatograms of chlorination TPs from batch experiment (upper part) vs. analytical standards (lower part) are shown, in column five important data of the analytical standards are illustrated.

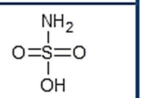
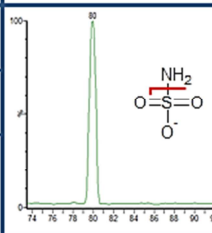
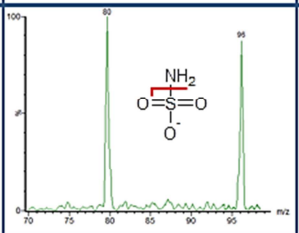
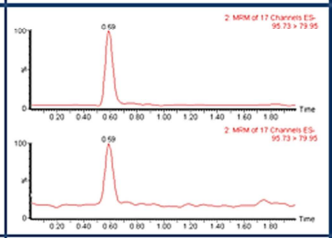
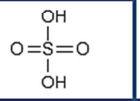
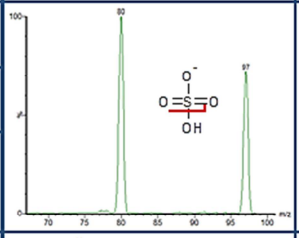
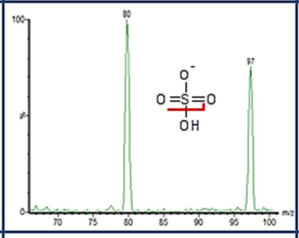
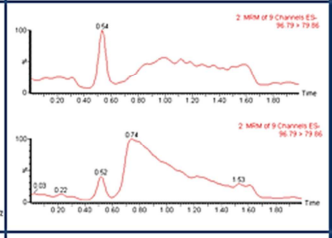
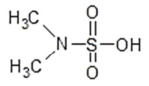
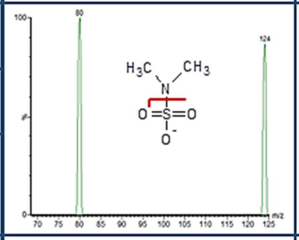
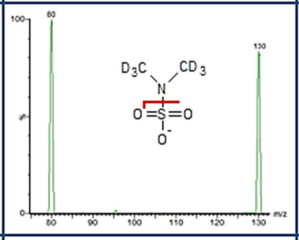
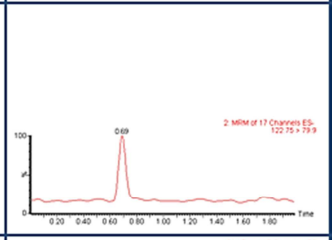
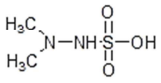
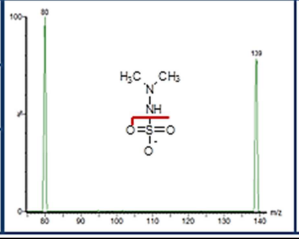
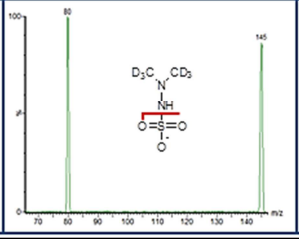
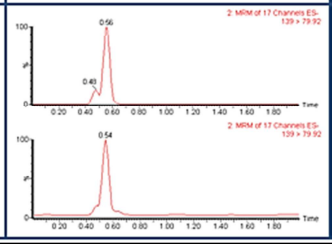
Proposed compound	MS/MS-Scan of DMS	MS/MS-Scan of D6DMS	SRM of standard / by-product	Used analytical standards
M97 H2NO3S 				Name: Sulfamic acid Ionisation: ESI- CAS number: 5329-14-6 Molecular mass (g/mol): 97.09 Mass [M-H]⁻: 95.73 Product ion: 79.95 / 63.80 Δ% retention time: 0
M98 H2O4S 				Name: Sulfate Ionisation: ESI- CAS number: 18785-72-3 Molecular mass (g/mol): 96.06 Mass [M-H]⁻: 96.79 Product ion: 79.86 / 63.90 Δ% retention time: 3.7
M125 C2H7NO3S 				Not available
M140b C2H8N2O3S 				Name: Dimetylsulfamoyl - Chloride Ionisation: ESI- CAS number: 13360-57-1 Molecular mass (g/mol): 143.59 Mass [M-H]⁻: 139.0 Product ion: 79.92 Δ% retention time: 3.6

Figure 5-8: Assumed empirical formulas, structures, and MS/MS spectra of chlorination TPs in ESI negative mode of DMS (left) and D_6DMS (right). In column four, the SRM chromatograms of chlorination TPs from batch experiment (upper part) vs. analytical standards (lower part) are shown, in column five important data of the analytical standards are illustrated.

In addition to the chlorination TPs, MS/MS-scans of DMS and D_6DMS were taken. In Figure 5-7 and Figure 5-8, similar MS/MS spectra of both substances are shown, except the mass shift of 6 Da. The resulting fragments m/z 45, 46, 63 80, 108 for DMS and m/z 50, 51, 63, 80, 114 for D_6DMS were equal to the previously detected and examined masses of chlorination TPs.

5.4.2.3 Structure verification

Subsequently, the predicted structures were verified in connection with the purchased analytical standards. Except for M60 and M125, for all chlorination TPs analytical standards could be obtained. For M140a/b dimetylsulfamoyl-chloride was obtained, which reacts in water by substitution of the chlorine atom by a hydroxyl

group to 2,2-dimethylhydrazinesulfonic acid. A LC-MS/MS SRM method was developed for the verification of TPs. The solved analytical standards (each 250 µg/L) were tuned automatically by Auto-Tune-Wizard to obtain optimal MS parameters. The chromatographic conditions and chlorination batch experiments were equal to the kinetic experiments carried out previously (Chapter 5.4.1.2.). The identification points of the formed degradation products were the precursor ion, two product ions and the retention time. The SRM chromatograms are illustrated in Figure 5-7 and Figure 5-8. All identification points were confirmed, the retention times of analytical standards and chlorination products differed hardly (deviations < 4%). The SDMH (M60) could not be detected in the last performed studies, because low concentrations were formed during the chlorination reaction and will not be considered in the further investigations. Based on the recorded data, a reaction pathway was proposed.

5.4.3 Reaction pathway and conversion rate

5.4.3.1 Reaction pathway

In Figure 5-5, it is illustrated that DMS was completely degraded within few seconds (t = < 0.5 min). The amine and amide group are attacked by hypochlorite because of their nucleophilic character. It is assumed that a DMS-Cl intermediate is formed, which quickly reacts further. The lower amount of DMS-Cl decomposes via hydrolysis to the main products DMA, sulfate, sulfamic acid and dimethylsulfamic acid (Figure 5-5: t < 20 min). This proposed reaction pathway is illustrated in Figure 5-9 as reaction pathway a. The larger amount of the DMS-Cl reacts fast over the ring formation between the amine and dimethylamine (reaction pathway b). This intermediate reacts by hydrolysis to 2,2-dimethylhydrazinesulfonic acid, while chloride is removed. 2,2-dimethylhydrazinesulfonic acid is known as an instable intermediate that reacts further via hydrolysis to UDMH and sulfate. UDMH is also known as instable intermediate and reacts further during the chlorination reaction mainly into dimethylamine [2]. This leads to an increasing amount of DMA after degradation of UDMH. This assumption was generated from the fact that the amounts of DMA and sulfate increased after the mentioned intermediates were

degraded (Figure 5-5; t ≥ 20 min). The proposed reaction pathway is illustrated in Figure 5-9 as reaction pathway b.

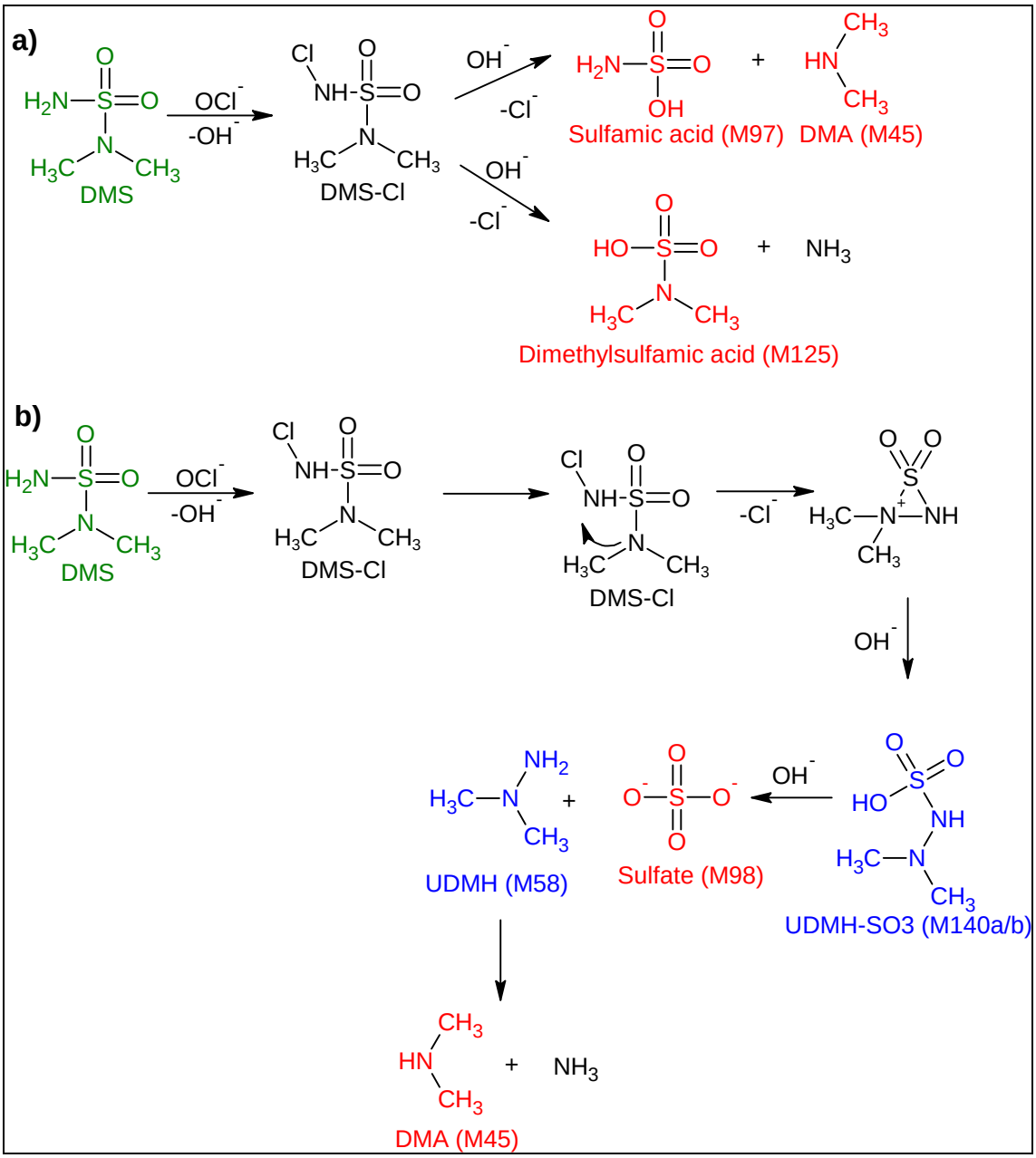


Figure 5-9: Two assumed reaction pathways of DMS chlorination by hypochlorite. Green structures show the DMS, black the assumed intermediates, blue the detected intermediates and red the detected main products.

5.4.3.2 Conversion rate

In the following experiments, the total conversion rates of the chlorination of DMS were examined. The determination was conducted with the established LC-MS/MS SRM method, which was used previously for the verification of TPs with analytical

standards (Chapter 5.4.2.3). The batch experiments were carried out for 24 h with a DMS concentration of 3 µg/L and 0.3 mg/L hypochlorite, respectively. The DMS concentration was chosen as low as possible, so that the relevant chlorination TPs could still be quantified with sufficient intensity (detection limit was ≤ 0.15 µg/L for all compounds). From the used DMS concentration of 3 µg/L (0.024 µmol/L), the following amounts were formed: 0.5 µg/L (0.011 µmol/L) DMA, 0.185 µg/L (0.0019 µmol/L) sulfamic acid and 0.57 µg/L (0.0059 µmol/L) sulfate. The concentration of other products was below of the LOQ and can be neglected. The investigation with drinking water samples (n = 3) in batch experiments, resulted for DMA in similar yields between 44 - 49%. The sulfate concentration could not be determined due to the high background concentration.

The chlorination of DMS led to the formation of 44 – 49% DMA, 8% sulfamic acid, and 25% sulfate. Due to their amounts, DMA and sulfate were the relevant degradation products during the chlorination of DMS. Due to the incomplete conversion rate, it is possible that further chlorination TPs are formed, which could not be detected with the used equipment. In a parallel research project by another group, the same TPs have been described [11].

5.4.4 Relevance of the formed TPs from DMS chlorination for drinking water treatment

Based on monitoring data, the average DMS concentration in ground water was 0.7 µg/L. Under the assumption that the concentration was not reduced by drinking water treatment and using the determined conversion rate, mean concentrations for DMA (c ≈ 0.34 µg/L) and sulfate (c ≈ 0.175 µg/L) were calculated. Because sulfate is a natural component of the groundwater with sulfate concentrations between 50 to 140 mg/L [12], it can rather be neglected as a chlorination TP from DMS. The polarity of DMA (K_{ow} = - 0.38) [13] makes this component a relevant factor for drinking water treatment. DMA is a chemical precursor for many products, with a production amount of 210,000 t/a for Western Europe [14]. The main application of DMA is the manufacture of N,N-dimethylformamide and N,N-dimethylacetamide as precursors for further chemical syntheses. Dimethylamine is also used as a precursor for the production of pharmaceuticals and insecticides [14, 15]. Moreover, dimethylamine is

formed as a metabolite in animals and plants, as well as in the metabolism of pesticides in soil [16]. Concentrations ranged from 0.1 to 0.55 µg/L in German rivers (Elbe, Neisse and Rhine) in 1994 – 1996; in Hesse rivers (Fulda, Werra, Kinzig, Lahn, Eder) in 2001 were in 16 of 22 samples DMA measured with concentrations from 0.15 to 2 µg/L, the remaining samples were below the detection limit of 0.15 µg/L [17]. In 1995 DMA concentrations up to 3 µg/L were reported for the Rhine [16]. For dimethylamine, no threshold limit values for drinking water but environmental quality standard (EQS) value of 10 µg/L exists. For DMA, acute toxicity values have been reported: rat oral test LD_{50} 698 µg/L [18], guinea pig oral test LD_{50} 40 mg/kg and rabbit oral test LD_{50} 240 mg/kg [19]. In several investigations no evidence of carcinogenicity or genotoxicity of DMA was detected [20, 21]. No long-term toxicity of DMA could be verified for rainbow trout below 0.6 mg/L [22]. The collected data can be helpful in assessing the relevance of formed DMA and sulfate during the chlorination of DMS containing drinking water. Based on data mentioned above (occurrence in environment and toxicological data), it can be assumed that the low concentrations (< 0.4 µg/L), which potentially may be formed from DMS via chlorination processes, can be classified as not acute toxic. However, further information is needed especially for the chronic influence of DMA on human health for a final assessment.

5.5 Conclusions

UPLC-ESI-MS/MS for the sensitive detection and complementary high resolution mass spectrometry for the elucidation of transformation products was successfully used to characterise reactions of DMS during chlorination. An isotopically labeled D_6DMS standard and the monitoring of the reaction time course was very helpful for the clarification of detection and structure elucidation.

During the chlorination reaction, several intermediates and main products were identified. As main transformation products in significant concentrations, dimethylamine and sulfate have been confirmed.

The results also showed, that dimethylamine and sulfate are less relevant for drinking water treatment, since their occurrence in the environment was at significantly higher

concentrations compared to the concentrations obtained in the chlorination reaction. The comparison with literature data showed furthermore that the amount of DMA formed by chlorination has no acute toxic potential. However, further information is needed for the chronic influence of DMA on human health for a final assessment.

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Chapter 6. General conclusion and outlook

The present thesis demonstrates three sensitive, accurate, and reliable analytical methods for the quantification of 29 polar pesticide metabolites at trace levels in different water bodies. All methods were validated and fulfil the requirements for the determination in trace range as well as for economic routine laboratory work. The method development included both the optimisation of chromatography and mass spectrometry. Through the selection of suitable column phase materials and gradients (on-column focusing), it could be shown that despite the use of reversed-phase and columns with small inner diameter, the LVDI is possible for the analysis of polar substances. Within mass spectrometric optimisation, it could be shown that careful selection of a suitable additive and its right concentration led to a significant increase of the LC-ESI-MS/MS signal response. In particular, this approach could be successfully used in Chapter 3, where post-column infusion of an additive to mobile phase after chromatography separation is applied and lead to signal enhancement up to a factor of 10. The second advantage of this procedure is, that the chromatographic separation is not hampered by the addition of an additive. The post-column infusion of ammonia as an approach to increase the ESI signal in positive ionisation mode was used for the first time, previously only TRIS for the negative mode has been known. This approach could be generalised to enhance the sensitivity of certain classes of compounds (amines and amides). A systematic study of matrix effects on the quantification of pesticide metabolites was conducted by different model samples that contain specific salts in concentrations that occur commonly in the environment (calculated from the concentrations of 600 real water samples). Partly, strong matrix effects were observed (deviation from the set concentration > 500%), which were substance dependent. The previous assumption could be confirmed, that the salts as matrix components in environmental samples cause matrix effects during the analysis of polar substances. Because of a lack of separation efficiency on reversed-phase for polar compounds, pesticide metabolites and salts elute together. Additionally applying post column infusion of an additive in the Chapter 3 led not only to an increased sensitivity but also to a partial reduction of matrix effects. This result was used successfully to reduce matrix effects during the

quantification of the two metabolites B and B1 of the pesticide chloridazon. The remaining matrix effects were compensated by isotope-labeled internal standards. Generally, this work confirms that the use of isotope-labeled internal standards were the most appropriate method to compensate matrix effects and the most feasible solution for economic routine laboratory work (Chapters 2 and 3). If no isotope-labeled standards are available, as an alternative the SAM is a suitable method to use instead. This method was used in Chapter 2 - 4 and has shown the successful use of the SAM to compensate matrix effects by quantification. However, this method is a time consuming, and labor-intensive approach for any economically working routine laboratory and should therefore only be used if carried out in an automated manner. Therefore, in Chapter 4, a fully automated SAM was developed and successfully used as a multi-component method for the determination of all investigated metabolites. The method development includes the automated sample handling and a work flow to calculate the concentrations as well as quality criteria. This method was validated and the reliability was tested in different water bodies. The developed analytical methods were successfully used in a national QA/QC round robin test in 2010 (AQS Baden-Württemberg – PT 2/10 special organic parameters in drinking water – “Nicht relevante PSM-Metaboliten und Glyphosat"). These analytical methods were applied to monitoring pesticide metabolites in waste water, surface water, ground water, and drinking water in the area around the Rhine and Ruhr. Evaluation of measured data shows that the majority of all samples contained at least one of the metabolites with a concentration higher than the limit of determination (> 30 ng/L). In more than 50% of all samples N,N-dimethylsulfamide, desphenyl-chloridazon, methyl-desphenyl-chloridazon, metazachlor-BH479-8, and S-metolachlor-CGA-380168 were determined. Flufenacet-M2, chlorthalonil-M12, metazachlor-BH479-4, and S-metolachlor-CGA351916 were found in more than 30% of all samples. Dimethenamid-P-M23, chlorthalonil-M5, metazachlor-BH479-9, and metazachlor-BH479-11 were not detected in any sample. These results showed that the quantity of detection can be correlated to the concentrations found within lysimeter tests carried out during the approval procedure for pesticides. In most cases, metabolites with higher concentrations in lysimeter tests are found in environmental samples. In Chapter 5, the degradation of DMS during chlorination with hypochlorite was investigated. As shown in previous monitoring data, the polar

metabolites have a great potential to reach ground water resources and cannot be removed effectively during drinking water treatment. If oxidation processes like ozonation or chlorination were used for drinking water treatment or safety disinfection, unknown TPs can be formed. Therefore, a workflow was developed for detection and characterisation of possible TPs exemplary for the DMS, for which it was known that the use of hypochlorite leads to degradation of DMS. Here, various identification strategies employing LC-MS and LC-MS/MS measurement modes were used to detect and to identify the chlorination TPs. Additionally, for effective identification and characterisation, kinetic studies, a deuterated standard D_6DMS and LC(HR)-MS were used, and finally the intermediates and main chlorination TPs were validated by analytical standards. As main chlorination TPs, dimethylamine and sulfate have been detected. Finally, estimation of relevance to drinking water treatment was conducted.

For future investigations, the following suggestions could be of interest.

Due to the limited monitoring data of up to now investigated pesticide metabolites and because their detection in all water compartments, a continuous monitoring of all metabolites is recommended. If a sufficient amount of data is available, a selection based on the exceeded HRIV values in ground water samples can be applied.

Meanwhile, for all pesticides approved in Europe, their metabolites and concentrations from lysimeter tests were published. For these pesticide metabolites, no real data about their behaviour in the environment exist. Therefore, the hitherto not determined pesticide metabolites – especially if observed in higher concentrations during lysimeter tests > 10 µg/L– should be also included for future monitoring programs.

Until now, the behaviour of examined pesticide metabolites within oxidative drinking water treatment, not regarding DMS, is widely unknown and needs further investigation. Adequate studies should be carried out as soon as possible for the pesticide metabolites with detections in drinking water > 0.1 µg/L.

As long as for most of the mentioned pesticide metabolites derived from lysimeter tests, no IS can be purchased, the automated SAM offers an adequate analytical

method to compensate potentially occurring matrix effects. Due to the previous mentioned limitation of the software, all analysed data must be exported manually for further evaluation. Here, the SAM could be optimised by a software solution, which could calculate the concentrations, and quality criteria fully automated. In addition, while post-running automated evaluation after each determination of a sample, the other four standard addition steps may be optional and thus can be skipped if no concentration higher than LOQ of the respective substance under investigation is detected.

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Kowal S, Balsaa P, Werres F, Schmidt TC (2009) Determination of the polar pesticide degradation product N,N-dimethylsulfamide in aqueous matrices by UPLC-MS/MS. Analytical and Bioanalytical Chemistry 395 (6):1787-1794.

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Curriculum Vitae

Der Lebenslauf ist in der Online-Version aus Gründen des Datenschutzes nicht enthalten.

Erklärung

Hiermit versichere ich, dass ich die vorliegende Arbeit mit dem Titel

„Determination of polar pesticide metabolites in aqueous matrices by LC-MS and investigation of occurring matrix effects"

selbst verfasst und keine außer den angegebenen Hilfsmitteln und Quellen benutzt habe, und dass die Arbeit in dieser oder ähnlicher Form noch bei keiner anderen Universität eingereicht wurde.

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